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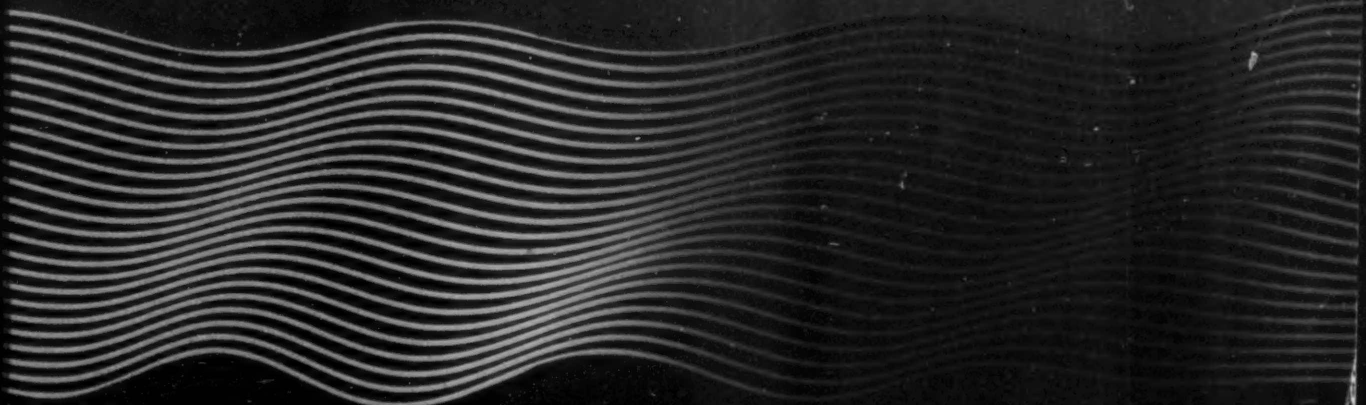

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Printed in Canada by: The Runge Press Limited, Ottawa

Imprimé au Canada par: The Runge Press Limited, Ottawa

Design: Ludvic Saleh & Associates, Ottawa

Conception graphique: Ludvic Saleh & Associates, Ottawa

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–1979.

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 48, No. 1, January 1991

Volume 48, nº 1, janvier 1991

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Postsmolt Atlantic Salmon (*Salmo salar*) in the Labrador Sea

D. G. Reddin and P. B. Short

Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, Nfld. A1C 5X1, Canada

Reddin, D. G., and P. B. Short. 1991. Postsmolt Atlantic salmon (*Salmo salar*) in the Labrador Sea. Can. J. Fish. Aquat. Sci. 48: 2–6.

In order to learn more about the existence, abundance, and distribution of postsmolt Atlantic salmon (*Salmo salar*) in the Labrador Sea, exploratory fishing was done with surface-set gill nets in early autumn of 1987 and 1988. In total, there were 468 salmon of various sea ages captured, of which 207 were postsmolts. The high catch rates compared with catch rates from Greenland suggest that the population of postsmolts in the Labrador Sea may be large. Postsmolts from rivers in Maine to Labrador were caught in the Labrador Sea, as indicated by Carlin tags and river age distribution from scale reading. The highest catch rates of postsmolts occurred in the Labrador Sea between 56° and 58°N latitude.

Afin de mieux connaître l'abondance et la distribution du saumon de l'Atlantique (*Salmo salar*) passé le stade de smolt dans la mer du Labrador, une pêche exploratoire au moyen de filets maillants déployés en surface a été effectuée au début des automnes 1987 et 1988. Au total, 468 saumons de différents âges de la phase en eau salée ont été capturés, dont 207 avaient passé le stade de smolt. Ces taux de capture sont élevés par rapport à ceux des pêches effectuées au Groenland, ce qui laisse penser que la population de saumons ayant passé le stade de smolt est importante dans le mer du Labrador. Des saumons ayant passé le stade de smolt provenant de rivières situées du Maine au Labrador y ont été pêchés, comme l'ont révélé les étiquettes Carlin et la distribution des âges établie par scalimétrie. Les taux les plus élevés de capture de saumons ayant passé le stade de smolt ont été obtenus entre les latitudes 56° et 58°N dans la mer du Labrador.

Received February 15, 1990
Accepted July 24, 1990
(JA477)

Reçu le 15 février 1990
Accepté le 24 juillet 1990

Much information has been published for most of the life history stages of Atlantic salmon (*Salmo salar*). However, little is known about the postsmolt stage, partly because there are no fisheries directly exploiting postsmolts and partly because little scientific effort has been directed at them (Caron 1983; Meister 1984; Dutil and Coutu 1988; Montevecchi et al. 1988). Allan and Ritter (1977) defined a postsmolt as a "juvenile salmon from the time that it leaves the river as a smolt until the onset of wide annulus formation on the scales at the end of the first winter in the sea." Knowledge of postsmolt biology including migration patterns, food resources, ocean distribution, and abundance would be very useful to resource managers, and scientists involved in Atlantic salmon enhancement and assessment programs. For example, knowledge of coho salmon (*Oncorhynchus kisutch*) biology during early marine life has been used to investigate growth rate selective mortality in the ocean off Oregon and Washington, USA, during years of low and higher survival (Fisher and Pearcy 1988); yet for Atlantic salmon a lack of knowledge of postsmolts would preclude such investigations. In this paper, we report observations on postsmolt salmon catches in the Labrador Sea from two research vessel cruises in the autumns of 1987 and 1988.

Methods and Materials

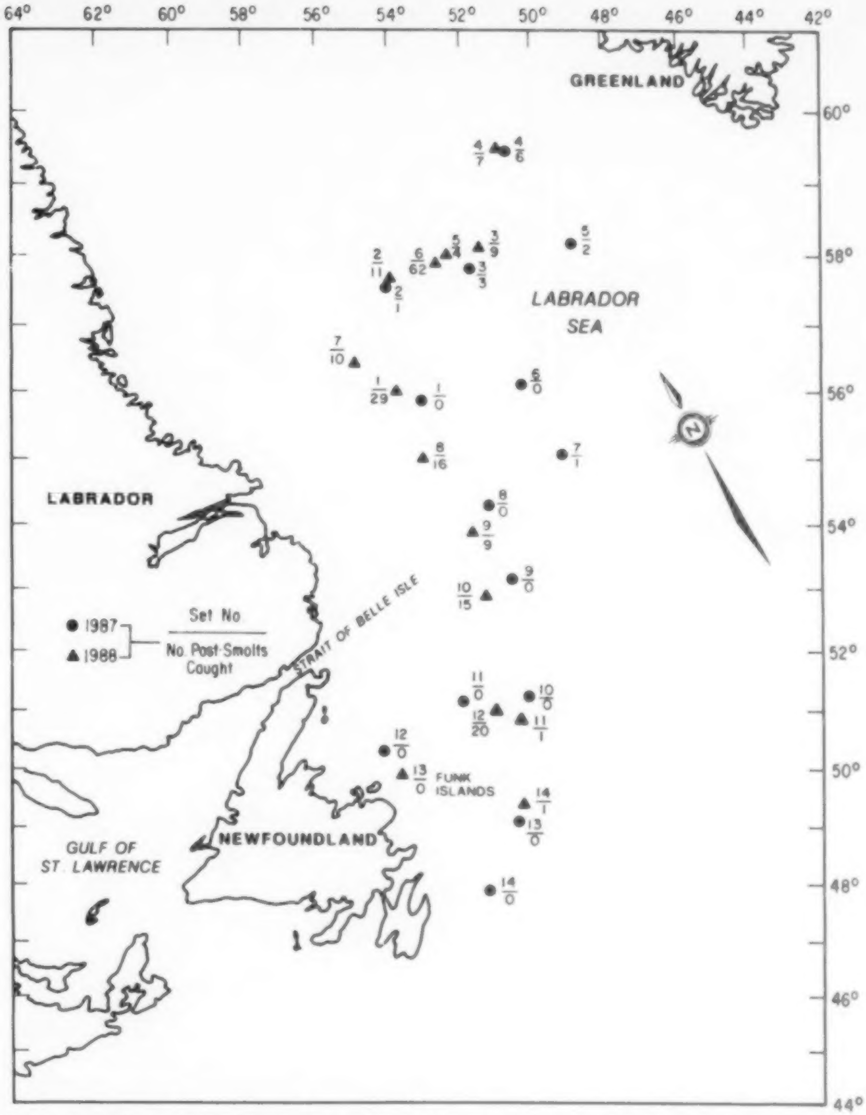
Fishing Techniques

Gill nets were fished on the surface in the Labrador Sea from the research vessel *Wilfred Templeman* from 24 September to 12 October 1987 and from 17 September to 6 October 1988 (Fig. 1). In 1987, 14 sets were made with up to 2743 m of monofilament gill nets set out in a string of basic units of five nets. Each basic unit consisted of five nets, each 46 m long, of mesh sizes 89, 102, 114, 127, and 140 mm (length of mesh opening). A basic unit was 230 m long, and an array of up to 12 units was fished at a time. In 1988, 14 sets were made with up to 3475 m of monofilament gill nets in a string of basic units of five nets. Each basic unit consisted of five nets, each 46 m long, of mesh sizes 77, 89, 102, 114, and 127 mm. A basic unit was 230 m long, and as many as 14 units were fished at a time. All nets were 3 m deep. Gill nets were set before dawn, depending on the weather conditions, and remained in the water for as long as possible, up to a maximum of 12 h.

Catch per unit effort (CPUE) data from the 1987 and 1988 surveys in the Labrador Sea were expressed as number of salmon caught per nautical mile-hour of gear fished. Effort expressed as mile-hours is the product of length of the nets fished and the time in hours that the nets were fished. Time (hours) was included in the CPUE statistic because of the variable length of time of the sets.

Data Collection

Live salmon in good condition were marked with modified Carlin tags, according to the technique described by Reddin (1984). All salmon, including those not suitable for tagging, were measured for fork length (nearest centimetre), examined for the presence of clipped adipose fins and other external marks, and scales were removed for age determination from the body position recommended in Anonymous (1984). In addition, all salmon which were unsuitable for tagging were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GREENLAND
LABRADOR SEA
LABRADOR
NEWFOUNDLAND
GULF OF ST. LAWRENCE
STRAIT OF BELLE ISLE
FUNK ISLANDS
● 1987
▲ 1988
Set No
No. Post-Smolts Caught
N
4/7
4/6
2/11
6/62
5/4
3/9
5/2
2/1
3/3
7/10
1/29
1/0
6/0
8/16
7/1
8/0
9/9
9/0
10/15
11/0
10/0
12/20
11/1
12/0
13/0
14/1
13/0
14/0

FIG. 1. Locations of sets during surface gill-net fishing for salmon in the Labrador Sea in autumn of 1987 and 1988.

weighed to the nearest 0.1 kg. Impressions of five scales from each fish were made on preheated plastic slides and projected onto the ground-glass screen of a microprojector at a magnification of 30×. The ages of these fish were interpreted using standard criteria (Anonymous 1984). Sea-surface temperatures were recorded for all fishing stations during the 1987 and 1988 surveys.

Results

Catch Rates

In the autumn of 1987, 136 salmon were caught, 13 of which were postsmolt salmon (Table 1). Postsmolt salmon were caught in 5 of the 14 sets and mainly in the northern area of the survey (Fig. 1). Catch rates per set for both postsmolts and older salmon were low, ranging from 0 to 1.53 postsmolt salmon per mile-hour with a mean of 0.23, and ranging from 0 to 3.51 salmon per mile-hour with a mean of 0.81 for older salmon. In autumn of 1988, 332 salmon were caught, of which 194 were postsmolt salmon (Table 2). Postsmolt salmon were caught in 13 of the 14 sets but, as in 1987, most postsmolts were caught in the northern areas (Fig. 1). Catch rates for both postsmolts and older salmon were higher than in 1987, ranging from 0 to 4.51 postsmolt salmon per mile-hour with a mean of 1.43, and ranging from 0 to 4.20 salmon per mile-hour with a mean of 1.55 for older salmon. Catch rates for postsmolts cannot be directly compared between the 1987 and 1988 surveys: some, if not all, of the difference in catch rates for postsmolt salmon between 1987 and 1988 may have been due to the smaller mesh gear fished in 1988. However, it was

TABLE 1. Summary of sets, salmon caught, and catch per unit effort from research survey in the Labrador Sea in 1987.

Set	Date	Position	Surface temp. (°C)	No. of postsmolts	No. of older sea age adults	Total	Catch per mile-hour	
							Postsmolts	All ages
1	28-09-87	55°53′N 53°05′W	6.9	0	7	7	0	0.57
2	29-09-87	57°29′N 54°01′W	7.2	1	15	16	0.19	1.24
3	30-09-87	57°46′N 51°35′W	7.1	3	27	30	0.44	1.77
4	01-10-87	59°25′N 50°48′W	6.8	6	6	12	1.53	1.22
5	02-10-87	58°08′N 48°53′W	7.4	2	5	7	0.67	0.94
6	04-10-87	56°09′N 50°13′W	7.2	0	50	50	0	3.51
7	05-10-87	55°04′N 49°19′W	8.2	1	9	10	0.38	1.53
8	06-10-87	54°17′N 51°17′W	6.3	0	3	3	0	0.49
9	07-10-87	53°08′N 50°40′W	6.2	0	0	0	0	0
10	08-10-87	51°14′N 49°58′W	5.1	0	1	1	0	0.07
11	09-10-87	51°04′N 52°00′W	7.2	0	0	0	0	0
12	10-10-87	50°15′W 54°12′W	5.8	0	0	0	0	0
13	11-10-87	49°06′N 50°17′W	6.6	0	0	0	0	0
14	12-10-87	47°56′N 51°02′W	7.9	0	0	0	0	0

apparent that postsmolt salmon were found in the Labrador Sea from mid-September onwards.

Linear relationships between postsmolt abundance and sea-surface temperatures ($R^2 = 0.004$, $P = 0.82$) and postsmolt abundance and latitude were not significant ($R^2 = 0.03$, $P = 0.55$). However, postsmolts were generally more abundant between 56° and 58°N latitude.

Tagging Results and Origin of Salmon

The river ages of the postsmolt salmon caught can be used to infer their origin because of the relationship of river age and latitude (Reddin and Porter 1988). Reddin and Porter (1988) reported, as have various other authors (Templeman 1967; Lear and Misra 1978), that more southerly rivers in North America typically produce younger river age salmon whereas more northerly rivers typically produce older river age salmon. For example, rivers in the Gulf of St. Lawrence produce salmon with river ages of 2 and 3 yr whereas rivers in Labrador produce salmon with river ages of 4 yr or more (Reddin and Porter 1988). In 1987, river ages of postsmolts caught in the Labrador Sea ranged from 2 to 7 yr (mean 3.9). In 1988, river ages of postsmolts caught in the Labrador Sea ranged from 1 to 5 yr (mean 3.0) (Table 3).

Of the 207 postsmolt and 251 older sea age salmon caught, 82 postsmolts and 53 older salmon, respectively, were tagged and released. No recaptures have been reported to date (9 March 1989). Six salmon were caught during the 1987 and 1988 research surveys that had been tagged with Carlin tags at various facilities in North America and released as smolts. Of these, two were postsmolts and four were one sea winter or older. Three of the older salmon and one postsmolt had been tagged and released into the Penobscot River, Maine, USA. The other adult salmon was tagged and released into Middle River, Nova Scotia, and the other postsmolt was tagged and released from the counting fence on Western Arm Brook, Newfoundland.

Discussion

The fact that many postsmolt salmon were found in the Labrador Sea within 4 mo of leaving their home rivers suggests that the Labrador Sea is an important nursery area for salmon during their early marine life. Also, the available evidence from tag recaptures and river age distributions shows that postsmolts from stocks over most of the salmon's range in North America can be found mixed together in the same area of the Labrador Sea.

More specifically, the results from our research cruises indicate that postsmolt Atlantic salmon were distributed over much

TABLE 2. Summary of sets, salmon caught, and catch per unit effort from research survey in the Labrador Sea in 1988.

Set	Date	Position	Surface temp. (°C)	No. of postsmolts	No. of older sea age adults	Total	Catch per mile-hour	
							Postsmolts	All ages
1	20-09-88	56°00′N 53°54′W	8.3	29	6	35	2.77	2.00
2	21-09-88	57°40′N 53°56′W	7.9	11	19	30	1.15	1.88
3	22-09-88	58°07′N 51°29′W	7.5	9	29	38	0.86	2.17
4	23-09-88	59°27′N 51°01′W	6.4	7	7	14	0.76	0.92
5	26-09-88	57°59′N 52°25′W	7.4	4	7	11	2.03	3.35
6	27-09-88	57°57′N 52°30′W	7.4	62	48	110	4.20	4.51
7	28-09-88	56°25′N 54°58′W	7.5	10	12	22	1.54	2.06
8	29-09-88	54°58′N 53°09′W	6.8	16	2	18	1.34	0.91
9	30-09-88	53°53′N 51°51′W	7.1	9	3	12	1.68	1.36
10	01-10-88	52°56′N 51°23′W	7.1	15	4	19	1.20	0.92
11	02-10-88	50°51′N 49°48′W	6.9	1	0	1	0.08	0.05
12	03-10-88	50°57′N 51°33′W	8.7	20	1	21	2.27	1.44
13	04-10-88	50°01′N 53°51′W	9.0	0	0	0	0	0
14	05-10-88	49°28′N 50°29′W	7.7	1	0	1	0.12	0.07

of the Labrador Sea in the autumns of 1987 and 1988. Postsmolt salmon were most abundant between 56° and 58°N. This is the first record of postsmolts caught at sea in the Northwest Atlantic Ocean other than in coastal areas. This information is consistent with the findings of Meister (1984) and Montevecchi et al. (1988) who speculated from tag recaptures that postsmolts from rivers in Maine, the Bay of Fundy, and the Atlantic coast of Nova Scotia migrate off eastern Newfoundland arriving in the vicinity of the Funk Islands (southern Labrador Sea) in early August. Caron (1983) and Dutil and Coutu (1988) concluded that salmon of some Gulf of St. Lawrence stocks exit the Gulf through the Strait of Belle Isle after remaining in the Gulf of St. Lawrence until late autumn. It is also probable that postsmolts spend the winter in the southern Labrador Sea, since high catch rates of one-sea-winter salmon have been made there in spring (Reddin 1985, 1988) and water temperatures in the Labrador Sea during winter are suitable for salmon survival (Saunders 1986; Reddin 1988). Therefore, we conclude that postsmolts from many of the Atlantic salmon rivers in North America can be found in the Labrador Sea in the autumn of the year they smoltified and may overwinter there.

Inasmuch as postsmolts were caught as far north as 58°N in mid-September, some postsmolts must have moved offshore into the Labrador Sea by midsummer. This is confirmed by the capture in the mid-Labrador Sea in September of tagged postsmolts from Western Arm Brook, Newfoundland, and Maine. Dutil and Coutu (1988) observed that some postsmolts remain in coastal areas in the Gulf of St. Lawrence as late as autumn before moving offshore whereas Meister (1984) and Montevecchi et al. (1988) concluded from tag recaptures that postsmolts from rivers in Maine, the Bay of Fundy, and the Atlantic coast of Nova Scotia arrived in the southern Labrador Sea in early August. Our data support the hypothesis of Montevecchi et al. (1988) and Meister (1984) rather than that of Dutil and Coutu (1988), although it is possible that postsmolts from some Gulf of St. Lawrence rivers behave differently from those from the Atlantic coast rivers.

It may also be that food resources and environmental conditions in the Labrador Sea influence postsmolt survival and growth as suggested by Reddin (1988). Scarnecchia (1983, 1984) demonstrated that for Icelandic stocks, water temperature, in early months that salmon are at sea, influences the salmon runs 1 and 2 yr later. Montevecchi et al. (1988) suggested that, in addition to causing significant mortalities in freshwater, avian predators may also cause significant mortalities of postsmolts at sea. More knowledge of these factors is important, as they would also influence the numbers of returning adults. Also, annual variations in these factors could influ-

TABLE 3. River age distribution of postsmolt and older salmon caught in the Labrador Sea in 1987 and 1988.

	Sea	River age								
Year	age	1	2	3	4	5	6	7	Unknown	Total
1987	0	0	4	2	2	2	2	1	0	13
	%	0	31	15	15	15	15	7	0	
	1	13	37	24	20	11	1	0	0	106
	%	12	35	23	19	10	1	0	0	
	2	0	1	1	1	0	0	0	0	3
	%	0	33	33	33	0	0	0	0	
	PS[b]	0	0	3	1	1	0	0	0	5
	%	0	0	60	20	20	0	0	0	
1988[a]	0	11	35	83	40	12	0	0	13	194
	%	6	18	43	20	6	0	0	7	
	1	19	44	30	26	10	0	0	3	132
	%	15	34	23	20	8	0	0	2	
	PS[b]	0	0	2	1	1	1	0	0	5
	%	0	0	40	20	20	20	0	0	

[a] Four salmon missing.
[b] Previously spawned salmon.

ence the grilse to multi-sea-winter salmon ratios of returns to home rivers, if postsmolts destined to return at different sea ages are distributed at different locations at sea.

Although it is not possible to estimate the number of postsmolts in the Labrador Sea, comparison of the mean catch rate from the 1988 survey with catch rates at Greenland where population sizes have been estimated can provide a rough approximation. A precise estimate of population size would require details on gear selectivity of all salmon age classes including postsmolts which are not available (Doubleday and Reddin 1981). Catch rates at Greenland have been expressed as the number of salmon per 100 standard nets and range from 10.2 salmon per 100 nets in 1970 to 111.0 in 1975 with a mean of 44.3 (Møller Jensen and Lear 1980; Reddin 1985). Catch rate for postsmolts in the Labrador Sea during our survey in 1988 was 21.3 per 100 nets. Estimates of population size at Greenland for the years 1969–85 range from 1.0 to 2.0 million salmon (Anonymous 1987). The fact that the mean catch rate for postsmolts in the Labrador Sea in 1988 was about 50% of the average catch rate at Greenland suggests that the number of postsmolts in 1988 in the Labrador Sea was quite high.

The marine life history of Atlantic salmon, especially the postsmolt stage, is an important area of research in which very little is currently being done. The fact that salmon from a wide geographic range in North America move into the Labrador Sea during the first few months of their marine life and may be found in abundance there may prove to be useful information for forecasting salmon returns well in advance of fisheries. Recently, predictions of multi-sea-winter components of salmon stocks have been considerably below forecasts for several North American rivers, and it is possible that the cause may be due to higher than normal mortality on postsmolts. Improvements to our knowledge of salmon marine life history, including the postsmolt stage, could provide answers to these and other questions. The data from our study suggest that salmon postsmolts are widely distributed over the Labrador Sea.

Acknowledgments

We thank Rex Porter, B. Dempson, D. Scarnecchia, and an anonymous reviewer for their helpful suggestions for improving the manuscript.

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A New Model of Particle Retention and Branchial Sieve Adjustment in Filter-Feeding Bream (*Abramis brama*, Cyprinidae)

Wim Hoogenboezem

Department of Experimental Animal Morphology and Cell Biology, Agricultural University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands

and

Limnological Institute, Tjeukemeer Laboratory, De Akkers 47, 8536 VD Oosterzee, The Netherlands

Jos G. M. van den Boogaart and Ferdinand A. Sibbing

Department of Experimental Animal Morphology and Cell Biology, Agricultural University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands

Eddy H. R. R. Lammens

Limnological Institute, Tjeukemeer Laboratory, De Akkers 47, 8536 VD Oosterzee, The Netherlands

and Arie Terlouw and Jan W. M. Osse

Department of Experimental Animal Morphology and Cell Biology, Agricultural University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands

Hoogenboezem, W., J. G. M. van den Boogaart, F. A. Sibbing, E. H. R. R. Lammens, A. Terlouw, and J. W. M. Osse. 1991. A new model of particle retention and branchial sieve adjustment in filter-feeding bream (*Abramis brama*, Cyprinidae). Can. J. Fish. Aquat. Sci. 48: 7–18.

A new model for filter feeding in bream (*Abramis brama*, Cyprinidae) is presented based on the three dimensional architecture of the branchial sieve. Transverse ridges on the upper surface of the gill arches form a system of channels in which food particles appear to be retained. These ridges are formed by a fleshy interconnection between the middle part of the gill arch and the bony parts of its gill rakers. Muscles attached to the rakers, present only on the lateral edge of the gill arch, indicate movability of the lateral bony raker element. If the fish is foraging on particles smaller than the channel diameter, movement of these gill rakers probably adjusts the sieve by reducing the channel diameter of the opposite channel. Selectivity of bream depends on available size classes of zooplanktons and changes in selectivity are attributed to adjustment of the branchial sieve. The channel model has been tested with feeding experiments and X-ray cinematography. The reconstructed paths of marked food particles show that particles follow the hypothesized path. Particle retention occurred mainly at the expected medial site of the arches. Our study strongly supports the channel model of particle retention.

On présente un nouveau modèle d'alimentation par filtration chez la brème (*Abramis brama*, Cyprinidae), modèle fondé sur la structure tridimensionnelle du filtre branchial. Des crêtes transverses situées à la face supérieure des arcs branchiaux forment un système de canaux qui semblent retenir les particules de nourriture. Ces crêtes sont formées par un pont charnu situé entre la partie centrale de l'arc branchial et les parties osseuses de ses branchicténies. Les muscles fixés aux branchicténies, présents uniquement sur le bord latéral de l'arc branchial, indiquent la possibilité de mouvement de l'élément racleur osseux latéral. Si le poisson se nourrit de particules dont le diamètre est inférieur à celui du canal, le mouvement de ces branchicténies ajuste probablement le filtre en réduisant le diamètre du canal opposé. La sélectivité de la brème dépend de la taille du plancton disponible et les modifications de sélectivité sont attribuées à l'ajustement du filtre branchial. Le modèle des canaux a été testé par des expériences d'alimentation et par radiocinématographie. La reconstitution du trajet des particules de nourriture marquées montre que ces dernières suivent le trajet prévu. La rétention des particules s'est surtout produite là où on l'avait prévue, c'est-à-dire au point médian des arcs branchiaux. Les résultats de notre étude appuient fortement le modèle des canaux dans la rétention des particules.

Received December 15, 1989
Accepted July 23, 1990
(JA407)

Reçu le 15 décembre 1989
Accepté le 23 juillet 1990

Among the European cyprinids, bream (*Abramis brama*, Cyprinidae) is one of the most effective feeders on both zooplankton and benthic chironomids (Lammens 1984; Lammens and Hoogenboezem 1991) and the dominant fish species in eutrophic lakes with limited macrophytes in northwestern Europe. Efficient exploitation of both benthic and pelagic prey seems contradictory. Hyatt (1979) described that branchial sieves of benthic feeders usually posses short gill rakers whereas zooplanktivorous species usually have elongated gill rakers. Although the branchial sieve of bream bears relatively short rakers as for example, compared with *Clupea* (Kirchhoff 1958; Gibson 1988), our experiments (Lammens 1985; Lammens et al. 1987) show unequivocally that bream forage successfully on zooplankton.

Size selectivity in filter-feeding fishes is usually related to interraker distances, which are taken as a measure of mesh size.

Predictions of size selectivity vary from relatively good (Wright et al. 1983; Drenner et al. 1984) to data showing hardly any relationship between size of ingested particles and interraker distances (Seghers 1975). Fishes may even retain particles smaller than predicted from their interraker distances (Gibson 1988). It was also found that removal of gill rakers of *Tilapia* had no effect on the size of ingested particles (Drenner et al. 1987). Thus, the actual sieving mechanism is more complicated than simple sieving through a filter (Rubinstein and Koehl 1977).

Until recently, particle retention in fish species with short rakers was thought to occur according to a simple two-dimensional interarch slit model. This model, first proposed by Zander (1906) and which seems to be generally accepted (Greenwood 1953; Matthes 1963; Lammens 1984; Northcott and Beveridge 1988), assumes narrow and more or less adjustable slits between adjacent gill arches forming the actual site of retention (Fig. 1A). These slits have a serrated appearance due to the interdigitation of gill rakers of both arches. Therefore, slit width between the arches should be related to the size of food particles. Thus, when feeding on small items, a narrow interarch slit is expected, but foraging on larger prey allows larger slits, enabling reduction of the sieve's resistance to water flow and increasing its capacity. The first attempt to test this hypothesis, by means of X-ray cinematography (Hoogenboezem et al. 1990), showed that the gill-arch slits are far too large (1.25–2.5 mm between arch 1 and 2) to explain the size of the ingested cladocerans (length 0.93 mm). A second condition for effective particle retention is constant interarch slits during sieving. However, these slits change constantly during filter feeding. (Hoogenboezem et al. 1990). Thus the simple two-dimensional model of particle retention appeared to be inadequate to explain the sieve mechanism.

In the present study a new model of particle retention and sieve adjustment is developed using the three-dimensional architecture of the branchial sieve. Food particles appear to be retained in channels on the upper surface of the branchial arch (Fig. 1B). We first describe the morphological and histological features of the branchial sieve and the results of feeding experiments which form the basis of the new model. We then describe a series of tests to which the model was subjected. Among these tests is the study by X-ray cinematography of the pathways of food particles during feeding.

Material and Methods

Bream for dissections and histological and feeding experiments were trawled in Tjeukemeer, a shallow, eutrophic lake in the north of The Netherlands.

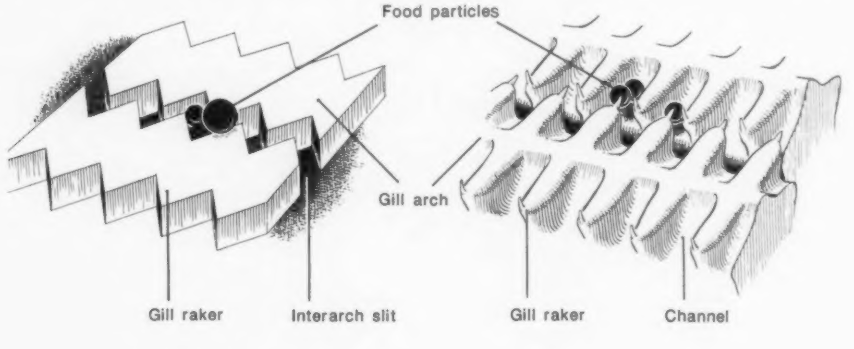
Morphology and Histology

Bream specimens (24.7, 27.0, and 33.0 cm standard length (SL)) for paraffin sections were decapitated immediately after capture. The complete branchial basket, including the palatal organ, was fixed in Bouin's fluid for 15 d (Romeis 1968). After sing with 70% ethanol, the material was decalcified in a mixture (1:1) of formic acid (100%) and ethanol (70%) for 30 d. The mixture was renewed every week. After a dehydration series over ethanol the material was impregnated over isoamylacetate with liquid paraffin in a vacuum stove at 60°C for 2 h and subsequently embedded in paraffin. Serial sections (5 μm) were cut, mounted on a slide, and stained after Crossmon (Romeis 1968).

For morphological studies, either fresh, frozen, or Formalin-fixed (4%) material was used.

Feeding Experiments

Bream of 18.0, 20.0, and 22.5 cm fork length (FL) were kept in an aquarium (180 × 60 × 60 cm) at 18°C and fed on living zooplankton for at least 1 mo before the experiments started. Four trials at high and three at low density were conducted. Before each experiment the fish were starved for 24–32 h. After adding freshly collected live zooplankton to the experimental tank, density and size distribution were determined by taking 12 water samples of 200 mL with a perspex tube at 12 sites. The 12 samples were filtered through an 80-μm plankton net and the zooplankton were preserved in 4% Formalin. The total volume of water was measured to the nearest 5 mL. The zooplankton lengths, excluding caudal spine and helmet, were measured to the nearest 0.02 mm using an ocular



A Interarch slit model

B Channel model

FIG. 1. (A) Scheme of the "interarch slit model" of particle retention. A serrated slit is formed by two adjacent gill arches. The supposed retention area is formed by the interdigitating gill rakers. (B) In the "channel model," food particles are expected to be retained in a channel formed by two adjacent gill rakers.

micrometer and were arranged in 15 length classes of 0.11 mm. This procedure was repeated after 2 h.

X-Ray Cinematography

Lateral and dorsoventral X-ray cine recordings were made of bream foraging in a perspex cuvette (1000 × 90 × 250 mm) provided with a measuring frame (Hoogenboezem et al. 1990). The experimental fish were marked with pieces of platinum wire (2–3 mm long, diameter 0.5 mm) in order to monitor the position and activity of the fish. Marks were placed on the skull (four markers), on the upper and lower jaws, pharyngeal jaw, and on the cleithrum. Branchial arches (I–IV) were marked anteriorly and posteriorly on the ceratobranchial bone. Markers in the palatal organ were implanted just underneath the eipithelium. Lateral and dorsoventral movies were recorded at 55–65 kV, 170 ± 10 mA, and object distance 85 cm with exposure times of 1–5 ms (see Hoogenboezem et al. 1990 for details). The films were recorded with an Arriflex 35-mm motion picture camera using Agfa Gevaert Copex Pan film at 25, 50, or 80 frames per second. The width of the pharyngeal slit, between roof and bottom of the mouth, was measured from lateral X-ray movies of a bream (25.5 cm SL) with marked gill arches and palatal organ. Paths of food particles in the mouth and branchial sieve were measured with marked *Daphnia pulex*. A small iron sphere (diameter 1 mm) was glued to the carapace (Bison Super glue) of a frozen *Daphnia* (length approximately 1 mm), which made it sink immediately. Two or three marked prey were mixed with ±50 unmarked dead *Daphnia* and placed on the bottom (area approximately 100 cm^2). This high number of *Daphnia* was needed to initiate feeding in the bream. The low number of marked specimen allows the recognition of a single intraoral path of a *Daphnia*. The fish (25.5 cm SL with two platinum markers per arch (I–IV) was recorded in dorsoventral X-ray movies while feeding on this mixture. Simultaneous video recordings were taken to observe the behaviour of the fish.

Location of Food Particles on the Sieve

The retention site of prey in the branchial sieve was determined in freshly collected fish specimens trawled from Tjeukemeer. Dissected branchial sieves were scanned with a stereomicroscope. Retained prey were identified, measured, and their exact location in the sieve recorded.

Results

Morphology of the Branchial Sieve

Macromorphology

The branchial sieve of bream consists of four paired gill arches, each bearing two rows of gill rakers (Fig. 2A). The fifth arches are modified into the characteristic cyprinid pharyngeal jaws (Sibbing 1982; Sibbing 1991a) and bear only an anterior row of gill rakers. As Sibbing (1988) found in carp (*Cyprinus carpio*), bream have a large muscular palatal organ forming the roof of the mouth and covering the entire branchial sieve, leaving only a narrow pharyngeal slit between branchial arches and palatal organ. The interarch slits constitute little to the total surface area of the branchial sieve (slits plus arches with channels). The area of arches I and II forms more than 75% of the total surface area of the branchial arches.

Micromorphology

Gill rakers — Each gill raker is composed of a cushionlike structure forming a transverse ridge and a distal bony part. The transverse ridge runs between the bony raker element and the central area of the arch. The central area is a low longitudinal ridge where both the lateral and medial raker cushions meet (Fig. 2B). The space between two adjacent transverse ridges forms a narrow channel, which is approximately 1 mm wide and 2–3 mm long in a 25-cm bream. Such channels appeared to be the site of particle retention (Fig. 2C). The bony part of the gill raker will further be referred to as the raker and the term channel will be applied to the space between two adjacent transverse ridges.

In the first gill arch, the medial channels are distinctly longer (70% of the arch width) than the lateral channels, and a distinct curvature can be seen in the former (Fig. 2B). In the second arch these differences are smaller. In arches III and IV the lateral and medial channels are equal in length and not distinctly curved. In the small area of the epibranchial bones (dorsal parts of the arches), transverse ridges, gill rakers, and channels are poorly developed.

The bony elements of the rakers rest with their base against the gill arch, run through the connective tissue cushion, and protrude from the distal parts of the transverse ridges. The rakers lying at the lateral side of the arch possess a long spine and have a spindle-shaped bony base, with their axis parallel to the arch (Fig. 3A). This form allows rotation of the raker during abduction or adduction through a pad of compact connective tissue (Fig. 4A). The medial rakers are somewhat shorter (Fig. 3B). They have broad flattened bases enveloped in connective tissue which is applied closely to the underlying bony ceratobranchials. These envelopes are interconnected and impede any movement in articulation (Fig. 4b).

Gill-raker muscles — Two striated muscles are attached to each of the lateral bony rakers of each gill arch. The first, the musculus abductor branchiospinalis, consists of a sheet of muscle fibers connecting the lateral gill raker, just above the raker base (Fig. 4A). Bijtel (1949) described this muscle for several fish species and called it the abductor muscle for the gill filaments. Since its actual function is probably abduction of the gill raker, musculus abductor branchiospinalis may be a more appropriate name. The second is the musculus interbranchiospinalis, a thin elongated muscle running between two adjacent gill rakers. It consists of a short muscle bundle at both ends tapering into a relatively long tendon, attaching at slightly different levels on the basis of both gill rakers (Fig. 4A). The function of this muscle is not known.

In contrast with the previous two muscles, the musculus constrictor canalis interbranchiospinalis is found on both the lateral and medial sides of the gill arch and is not attached to the bony rakers. The muscle originates under the ceratobranchial bone, runs over the ventral edge under the channel floor, and radiates into both channel walls. Here it forms a complex network of muscle fibers (Fig. 4A and 4B). Contraction of this muscle may cause deformation of the transverse ridges, affecting channel length and/or channel diameter.

Retention Site of Particles and Feeding Experiments

During the dissection and study of branchial sieves, we found prey items in the above-described channels. Also in sections for reconstruction work, we found parts of cladocerans in these channels (Fig. 2C).

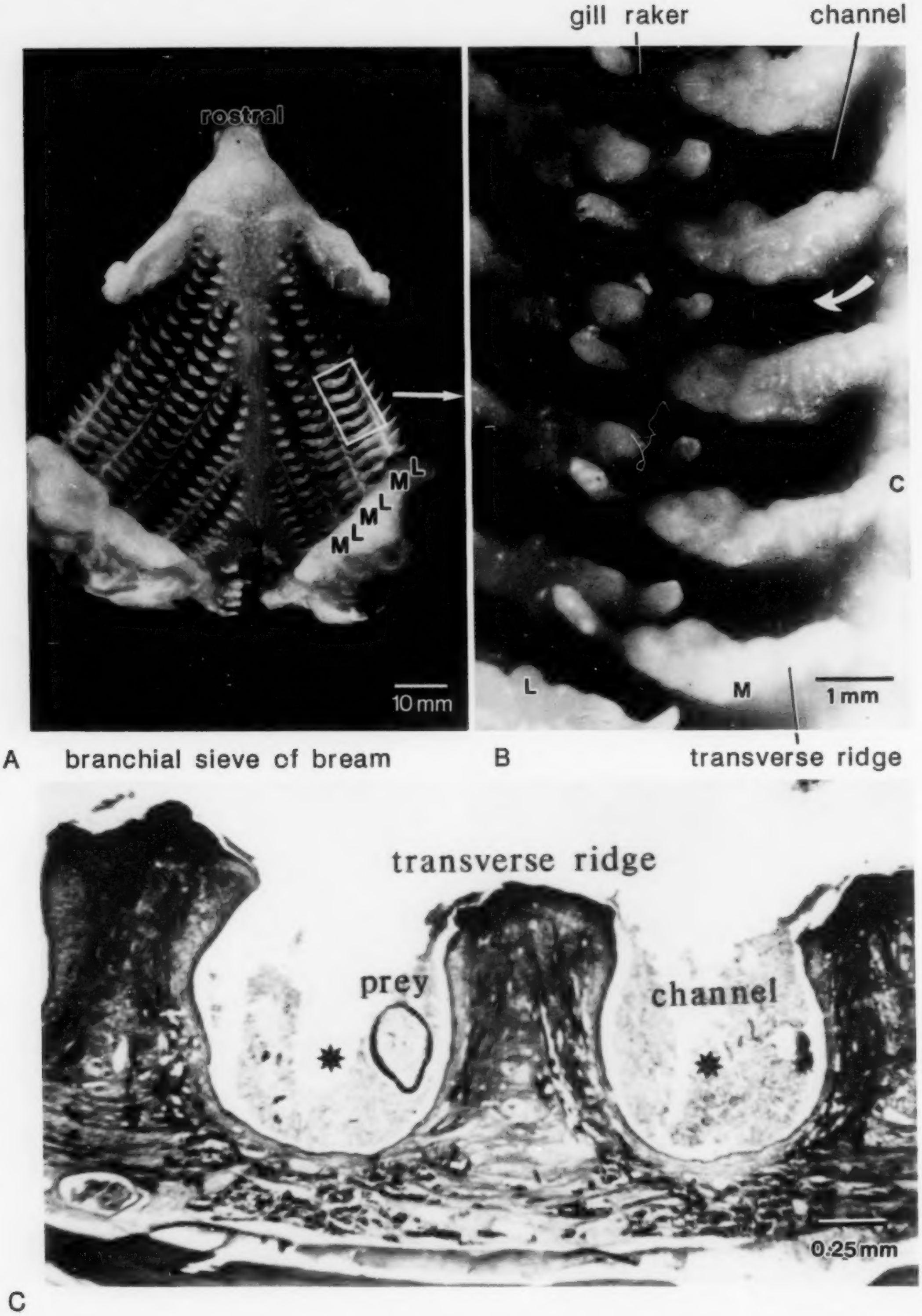

gill raker
channel
rostral
M
L
M
L
M
L
M
L
10 mm
C
L
M
1 mm
A branchial sieve of bream
B
transverse ridge
transverse ridge
prey
channel
0.25 mm
C

FIG. 2. (A) Dorsal view of the branchial basket of an adult bream, 37.4 cm SL. (B) Detail of two adjacent gill arches (right, arches I and II). L = lateral area; M = medial area; C = central area. Note the curved medial channels in the first arch (arrow). (C) Cross-section through two

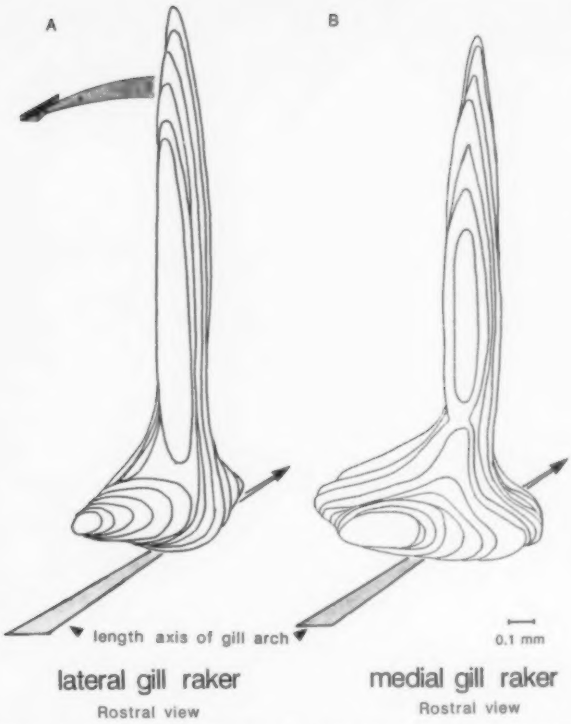


FIG. 3. Reconstructions (from serial sections) of lateral and medial bony gill-raker elements. Note the (A) spindle-shaped basis of the lateral gill raker and the (B) broad flattened basis of the medial gill-raker. The arrows at the bottom indicate the length axis of the ceratobranchial bone. The arrow at the top indicates the possible rotation of the movable raker.

Small bream (<15 cm SL are mainly particulate feeders and visual prey location is probably the decisive factor in food selection (Lammens 1985). Filter-feeding bream (mostly larger than 15 cm SL) take a series of snaps not directed at individual prey. Seven feeding experiments with bream (20 cm FL) were performed in aquarium tanks. In all experiments, the majority of the zooplankton consisted of cladocerans. The average composition was *Daphnia hyalina* (57.5%), *Bosmina coregoni* (22.4%), *B. longirostris* (8.1%), *D. cucullata* (0.2%), *Ceriodaphnia reticulata* (0.2%), *Chydorus sphaericus* (0.5%), and cyclopoid copepods (11.1%). Differential evasive behaviour of cladocerans and copepods could influence the selectivity (Drenner et al. 1978). In our experiments, 88% of the prey were nonevasive cladocerans, a suitable composition for unbiased tests on selectivity by bream. The selectivity for certain size classes of zooplankton depended on density and size distribution. In four trials (mean feeding time 2.0 h) at high density (416 ind/L), bream were strongly selective and ingested only prey larger than 0.9 mm (large/small prey ingestion ratio 10.6; Fig. 5A). The prey densities at t_0 and t_1 did not differ significantly for particles <0.9 mm (t-test: t = 0.612; n = 6), and for prey >0.9 mm the density after feeding was significantly lower than t_0 (t-test: t = 6.553; n = 8). Under conditions of low prey density (51.3 ind/L) (Fig. 5B), the same fish now included small prey (>0.5 mm) in its diet and the large/small prey ingestion ratio was 1.32. For particles <0.9 mm the observed difference was just below the significance level of 5% (t-test: t = 2.052; n = 6); the density of prey >0.9 mm decreased significantly (t-test: t = 6.098; n = 8) after feeding. Video recordings of the high-density trials showed gulping bream. In the environment the ratio of large to small zooplankters was approximately 1.5. Thus in the high-density experiments, small particles are also inhaled but they are not retained in contrast with what we observed in the low-density experiments with the same fish. Although small particles are ingested less efficiently, this selectivity is considered different from the results of high-density experiments (cf. Wright et al. 1983). This shift in size selectivity of bream most probably resulted from adjustment of the branchial sieve.

New Model of Particle Retention and Sieve Adjustment

From our observations we conclude the following: (1) the interarch slits are too wide for effective particle retention, and moreover, a mechanism of interdigitating gill rakers results in a nonhomogeneous filter system, e.g. a filter with different sized pores, in which the largest amount of water will go through the largest gaps (Hoogenboezem et al. 1990); (2) the morphology of the pharyngeal cavity, with the palatal organ as a roof and the branchial arches as a perforated floor, seems to be "designed" for water flow conduction through the channels; (3) food particles are captured in the described channels; (4) the results of our feeding experiments indicate an adjustable filter system in bream; and (5) the movable lateral gill rakers and the associated muscles may accomplish the observed filter adjustment.

Mechanism of Particle Retention

We developed the following conception of the mechanism of particle retention and branchial sieve adjustment in bream as follows. Ingested water including zooplankton flows through the narrow slit between palatal organ and branchial arches. Large particles (>3 mm) are retained in this pharyngeal slit. From the curved form of the channels (arches I and II) the flow of water containing smaller food particles is expected to run mainly through the medial channels towards the interarch slit (Fig. 6). Retention of intermediate-sized particles (1–2 mm) occurs in the channels on the branchial arches. The smallest ingested particles (0.5–1.0 mm) are retained only in medial channels with reduced mesh size. Effective reduction of the channel diameter is assumed to be achieved by insertion of the movable lateral rakers, of the opposite gill arch, into these medial channels. The channel opening is reduced to half its size when the raker insertion is centrally in the opposing channel. The effective diameter is in fact even slightly smaller if the width of the raker tip is taken into consideration.

According to this hypothesis the following is expected: (1) the pharyngeal slit between the palatal organ and the branchial basket is kept narrow during feeding, causing a parallel current along the gill arches. This improves water conduction through the channels because the total surface of the arches with

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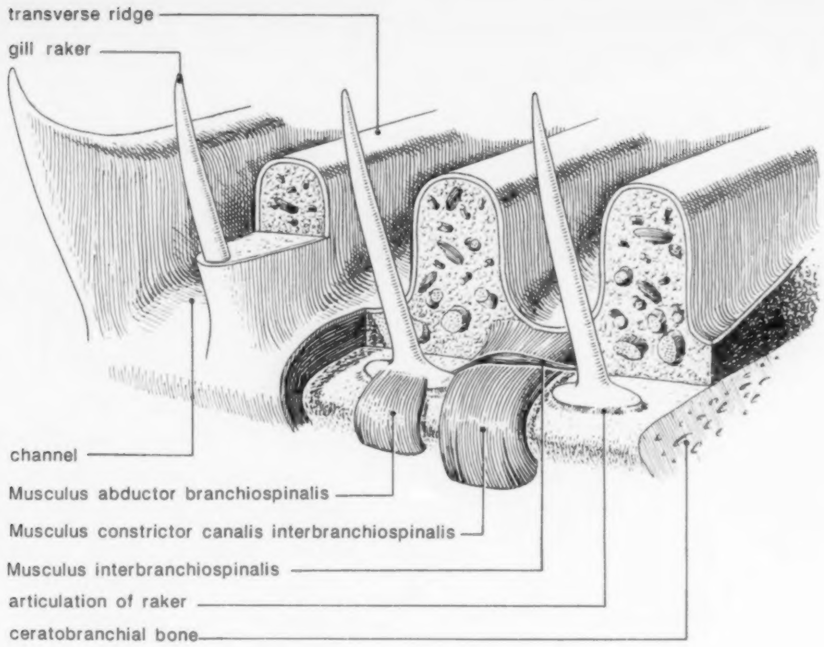
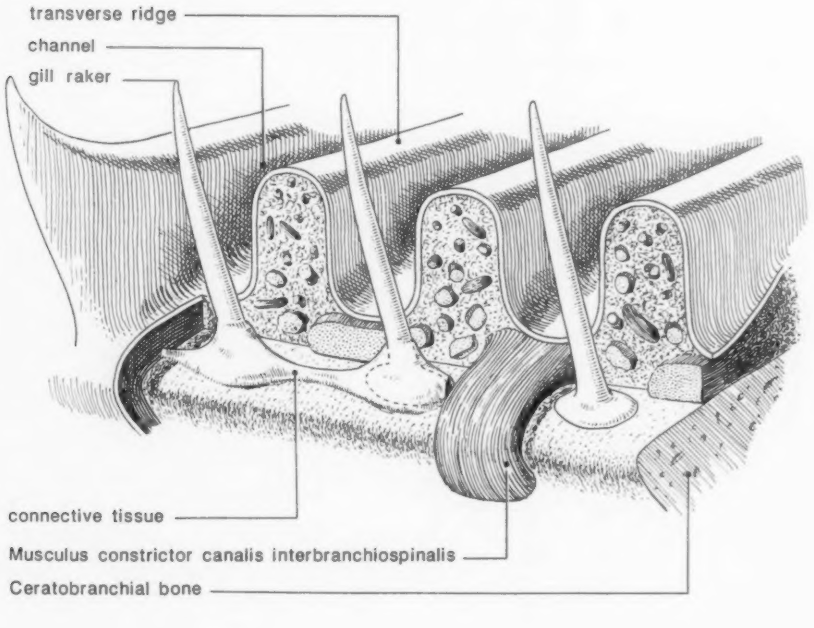
FIG. 2. (*Concluded*)
medial channels. Note the position of the prey (*Bosmina* sp.) between the walls of the channel and the place (asterisk) where the movable opposite raker is supposed to be during filtering.


transverse ridge
gill raker
channel
Musculus abductor branchiospinalis
Musculus constrictor canalis interbranchiospinalis
Musculus interbranchiospinalis
articulation of raker
ceratobranchial bone

A scheme of lateral view


transverse ridge
channel
gill raker
connective tissue
Musculus constrictor canalis interbranchiospinalis
Ceratobranchial bone

B scheme of medial view

FIG. 4. Scheme of the (A) lateral and (B) medial views of the gill arch indicating the positions of the gill rakers, muscles, and the connective tissue between gill-raker base and ceratobranchial bone.

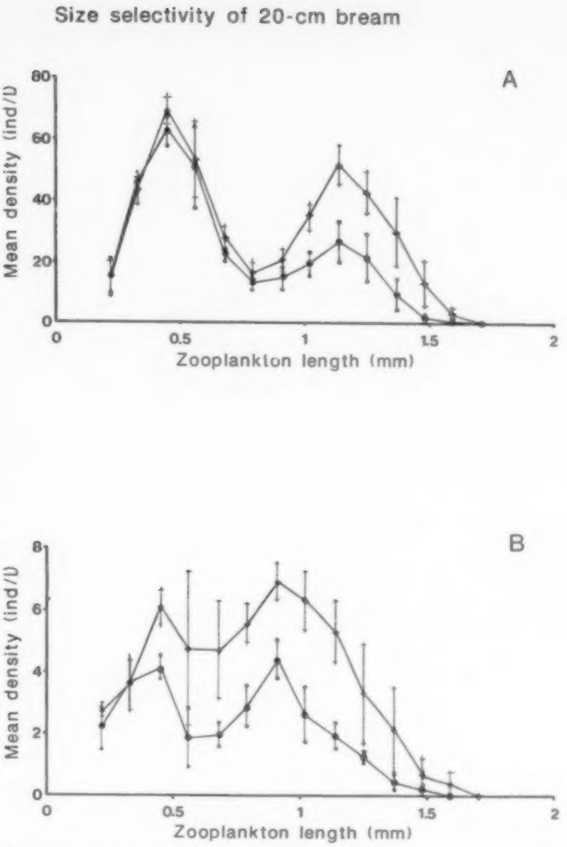


FIG. 5. Feeding experiments of bream (mean length 20.2 cm FL) foraging on living zooplankton. The mean densities of several experiments were plotted as a function of zooplankton size class. (A) Feeding at high prey density (mean = 416 ind/L ± SE $n = 4$) at the start (t_0) (+) and after 2.0 h (t_1) (●). (B) Feeding at low prey density (mean = 51.3 ind/L ± SE; $n = 3$) at the start (t_0) (+) after 2.16 h (t_1) (●).

channels far exceeds the area of the interarch slits; (2) food particles follow a path parallel to the arch and turn with a distinct angle (approximately 90°) into a channel where the particles are retained; (3) small particles are retained predominantly in the median channels because only these channels can be reduced in cross-sectional area by the movable rakers; (4) only two size ranges of food particles are ingested, one related to the channel diameter and the second to half the channel diameter; and (5) the width of the interarch slits may not exceed the length of the movable rakers.

Experimental Tests of the Model

Pharyngeal slit

According to the present model the vertical distance between the palatal organ and the branchial sieve is expected to be narrow during filtering. The variation of the pharyngeal slit was measured at several locations along the arch, from anterior to posterior (Fig. 7C and 7D, plots *A–E*). In a scene with chironomids as food items the maximal slit varies from 4.5 mm anteriorly to 3 mm posteriorly. While foraging on *D. pulex*, an even smaller maximal slit is observed (3 and 1.6 mm, respectively) (Fig. 7). The bream can open its pharyngeal slit considerably wider, up to 14 mm anteriorly at arch 2 (mean slit 11 ± 1.5 mm SD). Such wide slits were observed when bream fed on *Limax* sp., a slug measuring approximately 20 mm long and 10 mm wide.

Path of food particles

Small food items were expected to follow a path along the gill arch and turn sharply (approximately 90°C) into a channel where they would be retained. Such a path was followed using X-ray cine recordings of a marked bream foraging on marked *D. pulex*. Simultaneous video recordings showed that unmarked *Daphnia* were also inhaled, and no particle loss through the opercular slits was observed. The paths of 188 specimens of *D. pulex* during uptake and sieving have been studied in X-ray scenes. One hundred and forty-one prey specimens (75%) did not show a distinct angle in their path. The conglomerate of *Daphnia* and the iron sphere may be too large to enter the channel; the conglomerate is approximately 1 mm long, 1.5 mm wide, 0.4 mm thick. Retention probably occurred not in the channels, but in the pharyngeal slit between the palatal organ and the branchial arches (Fig. 8D). Carp similarly selects larger particles (Sibbing et al. 1986; Sibbing 1991b). Nevertheless, the paths of 47 ((25%) of the prey particles showed the expected flection clearly. Of these particles entering the channels, only 7 (15%) turned distinctly laterally and the rest, 40 (85%), turned sharply in the medial direction just before being retained in the channel. Some examples of these paths are plotted relative to the nearest marked gill arch (Fig. 8). Arriving dorsally near the sieve, the particles followed the branchial arch before turning into a channel (Fig. 8A–8C). As expected in a large filter area, compared with the relatively small volume of the buccal cavity, the velocity of marked particles decreased considerably when they reached the gill-arch area (mean velocity in buccal cavity 114.9 cm/s ± 62.8 SD; $n = 25$; filter area 11.1 cm/s ± 7.7 SD; $n = 29$). An example considered as pharyngeal slit retention is plotted in Fig. 8D. Two simultaneously ingested particles are seen to follow an arch and are retained without any curvature. In the trace in Fig. 8E, a particle above arch I suddenly turns laterally, up to 1.7 mm beside the branchial bone. Shortly after, it flows back in the mediocaudal direction, probably entering a medial channel to be retained at the medial edge of arch I.

Location of particles on the sieve

Zooplankton particles were found in the channels of the branchial sieve of 20–35 cm FL bream freshly collected from the lake. Because large amounts of mucus adhering to the gill arches often made tracing of food particles impossible, only fish lacking such large quantities of mucus were suitable for investigation. Therefore, only 23 fish were studied. In these specimens, 29 prey items were found in the channels. Of the 29 items, four *D. hyalina* and one *B. coregoni* were traced in lateral channels. Their lengths varied from 0.56 to 1.28 mm (mean length 1.03 mm ± 0.41 SD; $n = 3$). Five times more specimens of *D. hyalina* and *B. coregoni*, varying from 0.36 to 1.6 mm (mean length 0.75 ± 0.36 mm SD; $n = 24$), were found in the medial channels. The mean length of the particles found laterally is expected to be larger, but the low number of lateral retained prey precluded identification of statistically significant differences in prey size. Occasionally, particles were observed on the central area of the gill arch (mean length 0.67 ± 0.27 mm SD; $n = 4$).

If water is divided equally over both lateral and medial channels, it is expected that (larger) zooplankters are found in equal

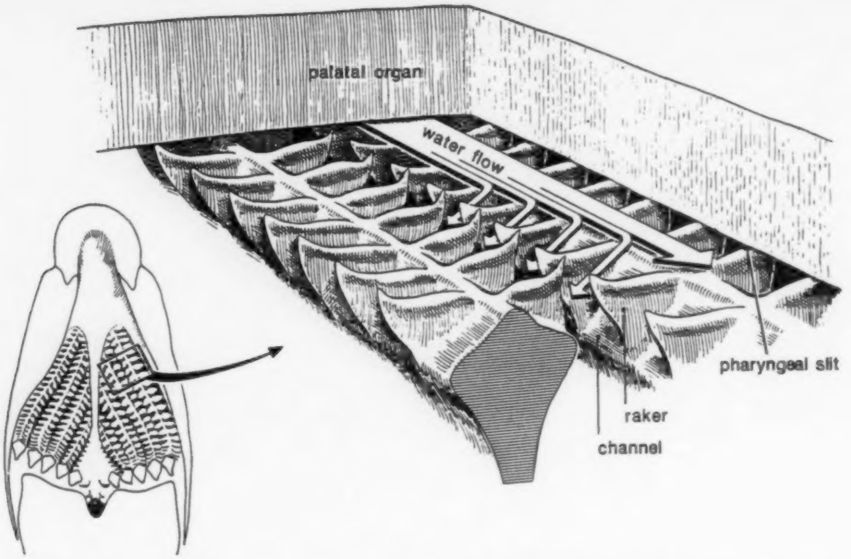


FIG. 6. Water flow (arrows) over the gill arch (right, arches I and II) and through the channels. Note the narrow pharyngeal slit; the water is forced to flow in a plane parallel to the arch and through the channels.

numbers laterally as well as medially (binomial distribution). However, we found a pattern significantly favouring the medial channels. Of 29 individual zooplankton traced in the channels, only 5 were found laterally. The lengths of the channels differ laterally and medially, especially in the first two arches. The mean asymmetry of the four gill arches was measured and resulted in different retention probabilities for particles laterally ($p = 0.56$) and medially ($p = 0.44$). According to a binomial distribution, with 29 prey items, the expected number for the medial channels (16.2 ± 2.7 SD) was significantly different from the observed (24) number (U-test: $U = 2.90$; $p = 0.0019$; $n = 29$) closely agrees with the percentage of marked *Daphnia* distinctly turning in the medial direction (85%) as observed in the X-ray cine recordings (see section on path of food particles).

Selectivity in feeding experiments

The feeding experiments (Fig. 5A and 5B) at high and low zooplankton density corroborate the two expected ranges of prey size. The size of ingested prey >0.9 mm is close to the diameter of the channels (0.82 mm) in 20-cm bream. The same fishes gulping at low food density retain all prey types >0.5 mm, coinciding with half of the channel diameter (0.41 mm).

Discussion

Three main aspects of the channel model (water flow, particle retention, and sieve adjustment) have been studied here. Our results support the validity of the model.

Water Flow

The architecture of the filter apparatus in bream appears to be designed to conduct water in a discrete direction. First, the palatal organ influences the flow distinctly by leaving only a narrow pharyngeal slit between the roof and floor. It was shown that only minor variations in slit width occurred (Fig. 7). Ingestion of larger prey induced clearly larger slits. During filter feeding the slit is considered small (1.6–3 mm) enough to conduct the water and particles parallel to the branchial arches into the channels.

Gill-arch movements have been measured in filter-feeding bream (Hoogenboezem et al. 1990). The interarch slits were wide relative to the daphnids ingested. Compared with the width of the gill arch, however, the interarch slits are relatively narrow. At maximal abduction of the gill arches the interarch slits occupy only 20% of the branchial surface. Thus, at least 80% of the surface of the sieve is occupied by the branchial arches. Consequently, the main bulk of water enters above the arches and will flow from the arch surface towards the interarch slits. The structure of the first arch is likely to conduct the water in the medial direction. The medial channels of the first arch are distinctly curved, from a longitudinal direction at the centre of the arch, towards a real transverse position at the medial end of the channel. Such a design would effectively conduct the water medially. Since the total area of the outlets of the medial channels is of the same order of magnitude as the area of the interarch slits, no major changes in the velocities of the streaming fluid will occur at this site. During filter feeding there may be, as in respiration (cf. Johansen 1977), a high resistance at the site of the gill filaments, when both functions are simultaneously executed. However, we do not know if this happens.

The elongated lateral gill rakers (first arch) are more or less paddle shaped and point more anteriorly than laterally in order to conduct the water medially. The well-developed lateral raker muscles in the first arch may serve in retaining such a spatial orientation during feeding.

The reconstructed paths of food items serve as indicators for flow directions in the branchial sieve. From particles small enough to enter the channels, 85% turned a medial direction and only 15% laterally. This confirms a mainly medial-directed flow over the sieve, as expected from morphological observations.

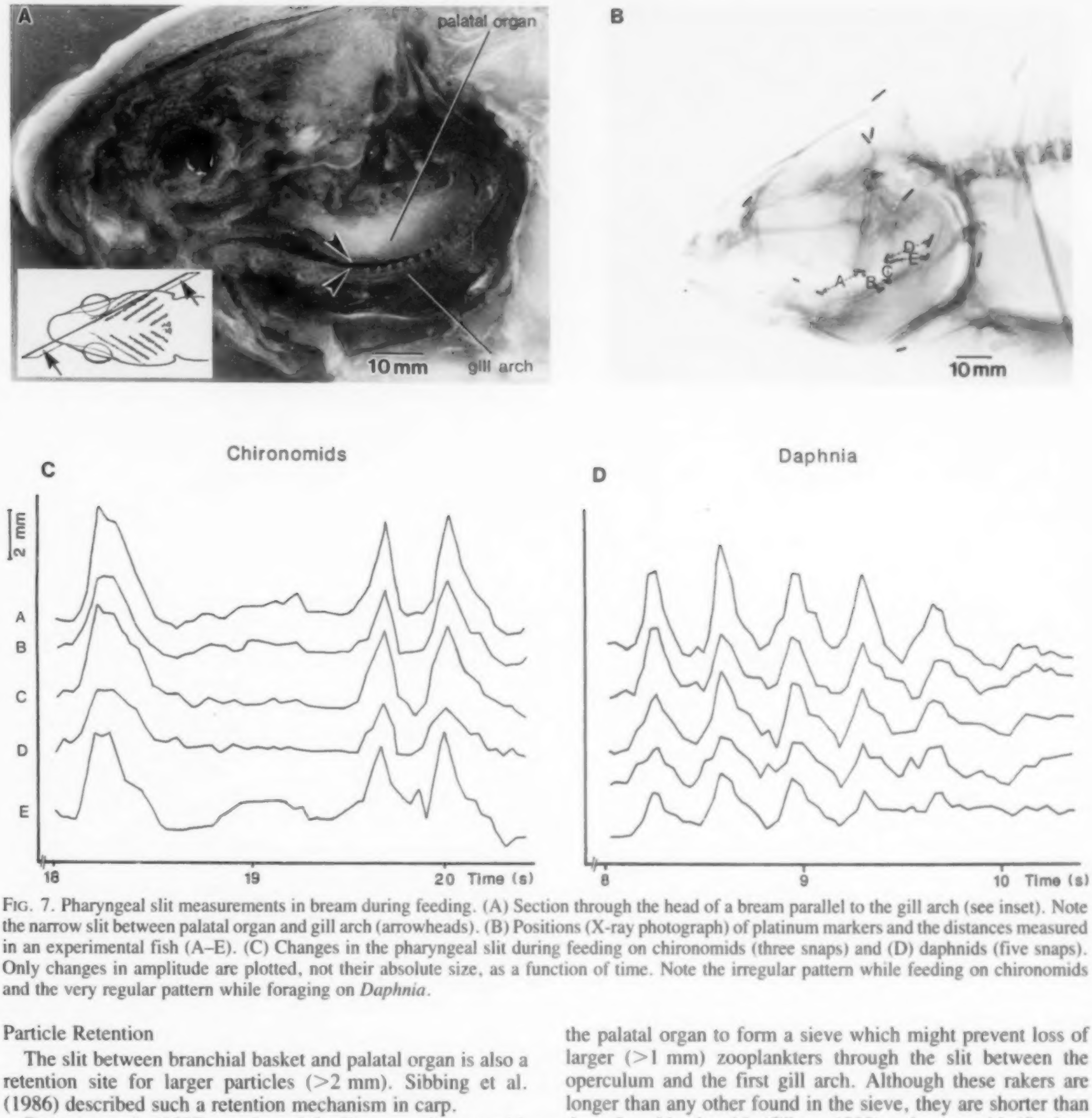


FIG. 7. Pharyngeal slit measurements in bream during feeding. (A) Section through the head of a bream parallel to the gill arch (see inset). Note the narrow slit between palatal organ and gill arch (arrowheads). (B) Positions (X-ray photograph) of platinum markers and the distances measured in an experimental fish (A–E). (C) Changes in the pharyngeal slit during feeding on chironomids (three snaps) and (D) daphnids (five snaps). Only changes in amplitude are plotted, not their absolute size, as a function of time. Note the irregular pattern while feeding on chironomids and the very regular pattern while foraging on *Daphnia*.

Particle Retention

The slit between branchial basket and palatal organ is also a retention site for larger particles (>2 mm). Sibbing et al. (1986) described such a retention mechanism in carp.

Drenner et al. (1987) conducted feeding experiments with *Tilapia galilaea*, feeding on a mixture of zooplankton and synthetic microspheres. Surgical removal of gill rakers and microbranchiospinae did not affect particle ingestion rates or selectivity. For the retention of larger particles (>0.5 mm) these results are not conclusive, since Drenner et al. (1987) did not include a description of the zooplankton decrease in these experiments. The largest particles they used (diameter 0.07 mm) were approximately 7 times smaller than the mean interraker distance (approximately 0.5 mm), while the space between two microbranchiospines was approximately 1.8 times the largest microsphere. This species may have a different retention mechanism for very small particles (<0.07 mm).

The lateral movable rakers of the first arch are distinctly the longest of the rakers in the sieve. Their tips probably reach to the palatal organ to form a sieve which might prevent loss of larger (>1 mm) zooplankters through the slit between the operculum and the first gill arch. Although these rakers are longer than any other found in the sieve, they are shorter than those found in clupeids (Gibson 1988) and coregonids (Seghers 1975). The discrepancy between interraker distance and the mean size of ingested particles as found in *Coregonus clupeaformis* (Seghers 1975) is probably caused by two factors. First, experimental fish were particulate feeders, feeding by directing their snaps towards individual visually selected prey. Second, almost all available prey were larger than the interraker distance; thus even incidentally ingested small prey were still large enough to be retained. In our view the zooplankton distribution was not appropriate to determine the retention capability of this species. Fishes in both families often have extremely long rakers on the first arch; in this type of fish the food particles will be strained mainly through this sieve of elongated gill rakers. The narrow pharyngeal slit in cyprinids does not require very long rakers and the elongated rakers of the first arch only pre-

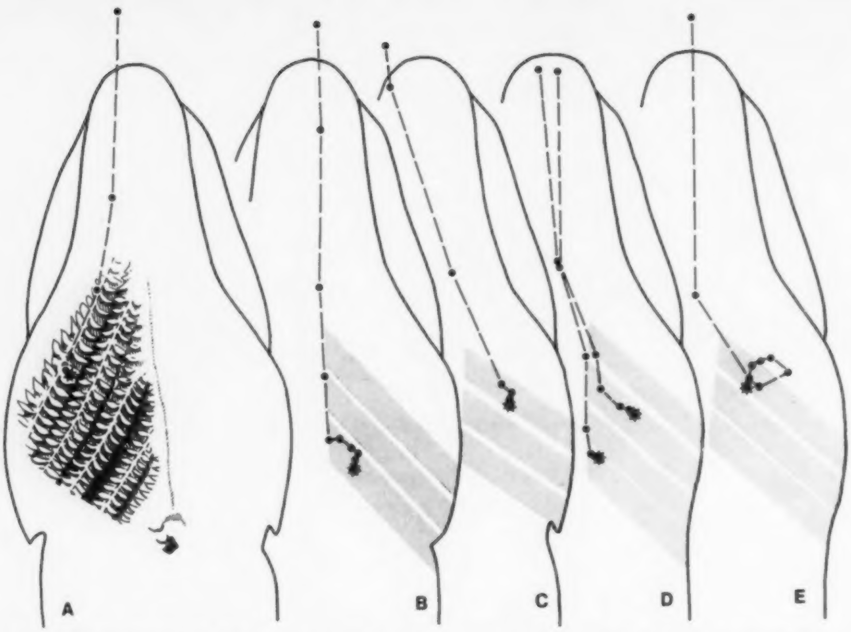


FIG. 8. Paths of marked food items (*Daphnia pulex*) in relation to the branchial sieve measured from X-ray cine recordings (see text for explanation). Solid circles represent subsequent film frames (A, 50 frames/s; B–E, 80 frames/s). Asterisks indicate the retention site of the particles. The position of the gill arches (shaded areas), reconstructed from gill-arch markers, is indicated for reference.

vent loss of large prey. The peculiar orbit depicted in Fig. 8E may show the effect of these rakers. The marked *Daphnia* only reached a maximum distance of 1.7 mm lateroanterior of the first arch, which is shorter than the mean length (2.41 ± 0.44 mm SD; $n = 27$) of the lateral gill raker of the first arch.

Small- and medium-sized cladocerans (0.36–1.6 mm) appeared to be retained in the channels, thus supporting the channel model of filtering.

Sieve Adjustment

Experimental conditions will influence the feeding mode, e.g. bream >15 cm are usually filter feeding at high food density, but at low density even large fish may feed as a particulate feeder (Lammens 1985; Lammens and Hoogenboezem 1991). Differences between these feeding modes involve the uptake of water and particles (Sibbing et al. 1986). In both cases the actual retention occurs after the prey have been inhaled. In our high-density feeding experiments, gulping bream inhaled but did not retain numerous small-bodied cladocerans. In the low-density trials, however, these small particles were inhaled and retained. This density-dependent selectivity is considered to indicate sieve adjustment. Two groups of particle sizes were ingested (Fig. 5), depending on density and size distribution of the food. At high density, fish retain particles >0.9 mm, but at low density they also retain particles >0.5 mm. The present channel model predicts two settings of the filter apparatus in bream. The first setting should be related to the channel diameter and the second is expected to be about half the channel diameter.

The smallest class of retained food items (0.5–1 mm) is assumed to be captured in diameter-reduced channels, although no direct observations of sieve adjustment by gill-raker activity are available. Channel reduction is probably achieved by insertion of a movable lateral raker into a medial channel of the adjacent arch (Fig. 9). The presence and insertion of the musculus abductores branchiospinalis at only these lateral rakers and their flexible cushions, combined with the spindle-shaped raker bases, strongly suggest lateral abduction of the raker. Electrical stimulation of the lateral raker area in anaesthetized fish (W. Hoogenboezem, pers obs.) showed distinct lateral depression of the raker tips. In the paddlefish (*Polyodon spathula*), movable gill rakers were described by Imms (1904). As in bream, the raker muscles in paddlefish interconnect the outside of the gill raker and the cartilage of the branchial arch. Imms (1904) suggested that the gill-raker muscles pull the rakers outward. He further hypothesized that elastic fibres between the gill raker and the branchial arch serve as antagonists and reposition the raker after its movement. The function of the musculus interbranchiospinalis is still unclear; it may aid in fine longitudinal adjustment to point the raker tip exactly in the centre of the opposing channel, when channel and raker are not properly aligned. Mechanoreceptors at these sites have been demonstrated by De Graaf et al. (1987). The third muscle system, the musculus constrictor canalis interbranchiospinae (lateral and medial), may by its contraction change the shape or length of the transverse channels, which may cause distal translation of the bony raker.

The varying interarch slit, as measured from X-ray cine recordings (Hoogenboezem et al. 1990), has a range perfectly fitting the channel model (Fig. 9). At maximal abduction of the gill arches, e.g. 2.5-mm slit between arches 1 and 2, the tip of the depressed movable raker is just in the outlet of the opposite channel. During gill-arch abduction the raker penetrates further into the channel (Fig. 9). The maximal effective interarch slit during filter feeding is related to the length of the gill raker. wider slits (>2.5 mm between arches 1 and 2) cause a decrease

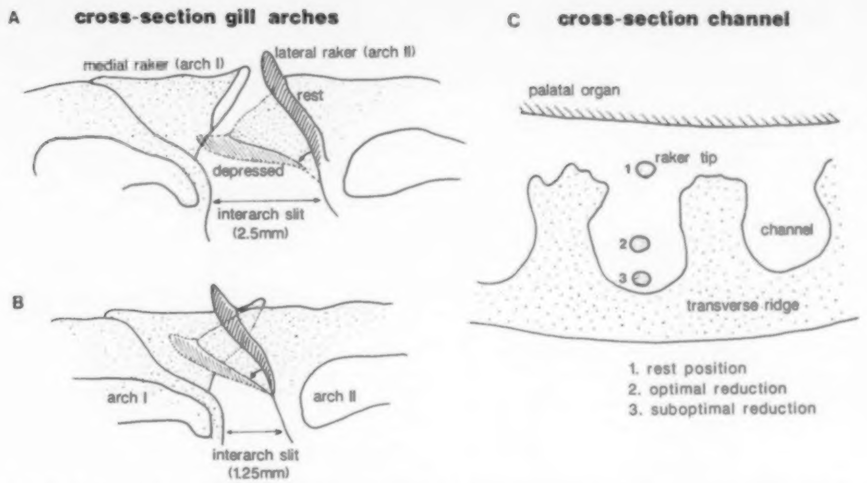


FIG. 9. Cross-sections through gill arches I and II (redrawn from photographs). (A) At interarch slit 2.5 mm, the (depressed) movable raker just reaches the outlet of the filter channel. (B) Minimal abducted situation. Over the whole compression phase, the raker can be kept in a fixed position relative to the channel, resulting in a constant mesh size during the sieve phase. (C) Possible raker-tip positions: 1, rest position of the raker not influencing the channel diameter; 2, raker reduces the channel to half its diameter; 3, the low position causes a heterogeneous filter with a narrow slit between the raker and channel bottom but large gaps between the raker and channel walls.

of retention ability, since the raker tip cannot be brought into the medial channel. Thus, effective retention in diameter-reduced channels is possible when the interarch slits do not exceed the movable raker length. Within these limitations, the channel filter-feeding model is independent of gill-arch movements, and a certain mesh size can be maintained during these actions. A constant mesh size during the compression phase of the oral cavity is important for effective retention, and for the effective use of the filter area as a whole. A filter with big holes will result in a major flow through these perforations and very little through the tinier meshes. When the model of interarch slits is considered, such a uniform filter is lacking (Hoogenboezem et al. 1990). In the present model the raker tip can also be inserted close to the bottom of the channel (Fig. 9). The area beneath the tip then represents a fine-meshed filter but the gaps on both sides of the tip remain large (approximately half the channel diameter). This results in a heterogeneous system within the channel. Particles even larger than half the channel diameter will escape through the enlarged gaps. Thus, to keep a more or less uniform mesh size, the raker is to be placed into the central area of the channel. Considering that channel diameters increase during the course of development (W. Hoogenboezem, pers. obs.), larger bream (>30 cm SL; channel diameter >1.27 mm) probably always feed with reduced channels on zooplankton.

The presence of mucus on the branchial sieve may implicate a "sticky filter" (Rubinstein and Koehl 1977). Changing size selectivity with changing zooplankton availabilities, however, cannot be explained with a sticky filter model. Besides, phytoplankton should then also be captured; however, there is no evidence for this, as determined by gut content analysis. Mucus may be a transport medium for small particles from the channels over the wide interarch slits to the chewing cavity and oesophagus. Two different types of oral mucus have been found in carp and related to intraoral food processing (c.f. Sibbing and Uribe 1985). Mucus also seems to play an important role in preventing loss of small particles in bream (W. Hoogenboezem and J. G. M. van den Boogaart, in prep.).

Integration of functional–morphological and ecological knowledge and techniques allowed us to develop and test hypotheses on the filter mechanism of the bream. The channel model not only describes the mechanism of particle retention, but it also determines the fraction of the food resources available to bream. Future comparisons of potential food resources for coexisting cyprinids may give clues for the success of bream in European eutrophic waters.

Acknowledgements

We are indebted to Drs. J. Vijverberg (Oosterzee), R. D. Gulati, and O. van Tongeren (Nieuwersluis) for their critical remarks on the various manuscript drafts, to Bert van den Bergh and Trudi Machielsen (Wageningen) for their assistance in the muscle reconstructions, and to Annet Kroon and Yvette van Vugt (Wageningen) for collecting data. Two anonymous referees are acknowledged for their constructive comments and Dr. J. Daly for improving the English text. We also thank Mr. W. Valen who drew some of the illustrations. Dr. G. Hoedemaker and Mr. C. Pootjes (Academic Medical Centre, Amsterdam) are acknowledged for making a Tacarno 35-mm movie analyzer available. The investigation was supported by the Foundation for Fundamental Biological Research (BION), subsidized by The Netherlands Organization for Scientific Research (NWO), Project 811-437-241B.

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Selection for Growth Potential among Migratory Brown Trout (*Salmo trutta*) Fry Competing for Territories: Evidence from Otoliths

Robert G. Titus and Henrik Mosegaard

Institute of Limnology, Uppsala University, Box 557, S-751 22 Uppsala, Sweden

Titus, R. G., and H. Mosegaard. 1991. Selection for growth potential among migratory brown trout (*Salmo trutta*) fry competing for territories: evidence from otoliths. Can. J. Fish. Aquat. Sci. 48: 19–27.

Otolith microstructure analysis was employed to determine differential size and growth characteristics between newly emerged migratory brown trout (*Salmo trutta*) fry which establish feeding territories and those which are forced to move downstream from the redd. Fry size at emergence was poorly predicted by otolith size, which precluded reliable back-calculation of fry size to investigate a possible size-selection mechanism. However, there was a clear-cut selection for individuals with significantly larger otoliths at emergence among fry which established territories in artificial stream sections. This selection pattern commenced with the onset of territorial aggression between fry. Fry which established territories to the end of the experiment had significantly higher initial otolith growth rates than emigrating fry, and their dry weights at the end of the experiment were well predicted by both otolith size at emergence and otolith size at the end of the experiment. Transparent zones in otoliths from downstream migrants suggested that these fry were starved; this was also supported by their declining dry weights over time. These results were explained as selection for greater growth potential as based on a proposed coupling between otolith microstructure formation and fish metabolism and on the behavioral ecology of migratory brown trout fry.

Des analyses microstructurales des otolithes ont été effectuées afin d'établir des relations entre les résultats de ces analyses et la taille et les caractéristiques de croissance d'alevins migrants nouvellement éclos de truite brune (*Salmo trutta*) qui défendent un territoire d'alimentation et de ceux qui sont forcés de se déplacer en aval du nid de fraie. La relation entre la taille des otolithes et la taille à l'émergence des alevins étaient faibles, ce qui rend impossible un calcul à rebours fiable de la taille des alevins pour vérifier l'existence d'un mécanisme de sélection suivant la taille. Cependant, parmi les alevins défendant un territoire dans des tronçons artificiels de cours d'eau, il y avait une sélection marquée de ceux ayant à l'émergence de plus gros otolithes. Ce mécanisme de sélection s'exerce dès que les premiers comportements d'agression des alevins les uns envers les autres, comportements liés à la défense des territoires, débutent. Les taux de croissance initiaux des otolithes des alevins ayant défendu un territoire jusqu'à la fin de l'expérience étaient nettement supérieurs à ceux des alevins ayant émigré, et il y avait une bonne relation entre leur poids sec à la fin de l'expérience et la taille de leurs otolithes à l'émergence et à la fin de l'expérience. La présence de zones transparentes sur les otolithes des alevins ayant migré vers l'aval laissent entendre que ces derniers ont manqué de nourriture; cette conclusion est étayée par le fait que leur poids sec allait en diminuant avec le temps. Ces résultats s'expliquent par l'existence d'une sélection favorisant les individus à plus grand potentiel de croissance comme le démontrent les rapports existant entre la formation microstructurale des otolithes et le métabolisme des poissons, de même que l'écologie comportementale des alevins migrants.

Received March 14, 1990
Accepted July 24, 1990
(JA502)

Reçu le 14 mars 1990
Accepté le 24 juillet 1990

Brown trout (*Salmo trutta*) fry occur in high densities upon emergence from gravel redds in late spring; however, mortality rates are high during the first summer and fry populations are regulated to much lower densities by autumn (Allen 1951; Le Cren 1973; Mortensen 1977a, 1977b, 1977c; Elliott 1984a). This decrease in population size occurs as fry establish and defend feeding territories in the stream (Le Cren 1973), their territoriality being mediated through intraspecific aggression (Kalleberg 1958). A social hierarchy is formed where dominant fry generally assume the energetically most profitable stream positions (focal point in low-velocity current to minimize energy expenditure on swimming, adjacent to a swift current where invertebrate drift rate is high; Fausch 1984). Some subordinate fry assume suboptimal feeding positions (sheltered positions away from faster currents), although most are eventually forced to move downstream, often to die (Elliott 1986).

What are the differential characteristics between individual fry which establish territories in the immediate redd area and those which move downstream? Inferences from previous studies suggest that size or growth selection may be a mechanism involved in the initial population regulation and spatial structuring of brown trout fry populations. For example, relative to fry which establish territories, downstream-moving brown trout fry have lower activity levels (Heland 1980a, 1980b), are smaller in size (Heland 1980a; Ottoway and Forrest 1983), and are in a poor nutritional state or moribund (Elliott 1986).

Our objective was to apply otolith microstructure analysis to determine differential size and/or growth characteristics between newly emerged fry of migratory (anadromous) brown trout which establish territories and those which move downstream. Otoliths may be used to back-calculate fish body sizes at a common previous time, e.g. to detect size-selective mortality following emergence. However, as a prerequisite, there must be a strong correlation between otolith size and body size at the previous time in question in order to make accurate back-calculations (e.g. West and Larkin 1987; but see also Mosegaard 1990). Because otolith growth rates appear to be coupled to fish metabolism (Mosegaard 1986; Mosegaard et al. 1988), comparative analysis of otolith sizes and increment formation rates may reveal differences in the metabolic performance of individual fry. Daily growth rings in the otoliths of young fishes also provide a record of individual growth histories and reveal the occurrence of stress events, such as starvation (e.g. Rice et al. 1985; Mosegaard 1986).

Materials and Methods

Experimental Facilities

Experimental work was conducted at the National Swedish Board of Fisheries' Research Station at Älvkarleby (central Sweden on the River Dalälven). Artificial stream sections were constructed from fiberglass hatching troughs measuring 2.2 m long × 0.4 m wide × 0.2 m deep (surface area per trough = 0.88 m^2). Seven troughs were arranged sequentially in a spiral pattern on platforms such that the first trough was at a basal height of 1.1 m and each subsequent trough was 14 cm lower; this created a gravity-driven flow gradient through the troughs. The troughs were linked with plastic ventilation hose (diameter = 10 cm) and river water entered the first trough through a large faucet. The first trough was not used for experimentation but served to baffle the incoming flow. The remaining troughs were numbered from 1 to 6, beginning with the most upstream one.

A styrofoam wall was inserted near the upstream and downstream end of each trough to create a waterfall to reduce turbulence and current velocity further. In between the waterfalls, the bottom of each trough was covered with an 8-cm-deep layer of mixed gravel (diameter = 10–60 mm), overlaid with cobbles (diameter = 10–15 cm) and gravel mounds.

Water depth (mean ± SD) between cobble groups and on gravel mounds was 10.0 ± 1.0 and 5.0 ± 0.6 cm, respectively. Middepth current velocity (mean ± SD) between cobble groups and on gravel mounds was 6 ± 5 and 10 ± 2 $cm \cdot s^{-1}$, respectively, and was 17 ± 7 and 25 ± 4 $cm \cdot s^{-1}$ on upstream and downstream waterfalls, respectively. Total discharge was 1.7 $L \cdot s^{-1}$.

Immediately below the upstream waterfall was a plastic emergence basket (30 × 20 × 11 cm) which was submerged in the gravel. On the sides of the basket were 7-mm slits through which emerging fry could swim. Perforated-plate screens (circular perforation diameter = 2 mm) were slanted into the downstream end of each trough below the waterfall and served not only to capture downstream-moving fry but to also isolate fish in each trough. The area between the downstream waterfall and perforated-plate screen was referred to as the downstream "trap" because brown trout fry within the size range encountered during this experiment were unable to swim upstream over the waterfall.

The experimental trough system was contained within a hatchery basin where only a roof provided shelter. Consequently, air temperature and light in the experimental area were essentially the same as natural ambient conditions.

Experimental Fish

Alevins (yolk sac fry) of the Dalälven stock of migratory brown trout were obtained from the research station in early May 1987. These alevins were the progeny of a single pair of wild parents; thus, between-female variation in initial sizes of offspring was eliminated, and the experimental populations simulated natural fry populations from single redds. The alevins were held at about 4°C and in total darkness where they developed to preemergence stage fry with yolk sacs nearly consumed.

On 27 June 1987, the fry were acclimated to experimental temperature (14°C) and then introduced into the emergence baskets between 1800 and 1845 on 28 June 1987. Total length (TL, mean ± SD) and dry weight (DW, mean ± SD) just before the start of the experiment were 27.1 ± 0.8 mm and 0.015 ± 0.003 g, respectively ($N = 32$). The mean proportion of yolk to tissue dry weight in emerging fry was 4.6% (SD = 3.8%, $N = 37$).

Experimental Design and Procedure

The fry were introduced at initial densities of 68 and $136 \cdot m^{-2}$, with three replicates at each density (these densities were selected as part of a concurrent study on density effects on population regulation, the results of which will be reported elsewhere). Troughs 1–6 were grouped into three blocks of two troughs each with regard to their placement in the hatchery basin and the resultant relative ambient light intensities in each block. Flips of a coin for each block randomly selected troughs 1, 3, and 5 to receive an initial density of 136 $alevins \cdot m^{-2}$ and troughs 2, 4, and 6 to receive an initial density of 68 $alevins \cdot m^{-2}$.

Once introduced, fry emerged from the baskets and dispersed throughout the troughs at will. The downstream traps were checked with a small dip net twice daily (~0700 and 1900) until experimental day 7, after which encounters of fry in the traps were so infrequent that trap checks were only made once daily (~1500). Trap-caught fish were measured for total length and then individually frozen in water in 1.5-mL plastic capsules for later processing.

Observations (mean ± SD period = 39 ± 11 min) were also made twice daily through the first 7 d of the experiment to follow qualitatively the development of fry territoriality. Of particular interest was the time after emergence when aggressive interactions between fry first began to occur.

In addition to feeding on juvenile insects that colonized the troughs via the water source, the brown trout fry were fed zooplankton on a drip system with a 15-min dose on every hour and a 2-h dose from 2100 to 2300 and from 0200 to 0400. Rations were in excess of the maximum that could be consumed because large quantities of zooplankton were always captured in a small net (mesh size = 250 μm) placed at the outlet of trough 6 during feeding pulses.

The experiment lasted for 28 d (28 June 1987 to 26 July 1987) during which time water temperature in the troughs gradually increased from about 14 to 21°C. The experiment was concluded by netting the remaining brown trout from the troughs.

Laboratory and Data Analyses

Emigration rates between initial densities were compared by use of chi-square analyses on the mean number of fry at each density emigrating into the traps during each sampling time interval. There was no significant difference in emigration rates between densities ($\chi^2 = 17.8$, df = 12, $p > 0.10$). This allowed us to combine emigration rate data from both initial densities in order to determine four main periods in the population regulation process, which in turn facilitated preplanned comparisons of fry groups between periods. Period 1 began at the start of the experiment and continued until aggressive interactions between fry were first observed, at which time period 2 was initiated. To determine the division between periods 2 and 3, finite emigration rates (*E*) were plotted against time by use of the equation

$$E = \Sigma\{(N_{jt_{i+1}} - N_{jt_i}) \cdot N_{jt_i}^{-1}\} \cdot (t_{i+1} - t_i)^{-1}$$

where *N* was the total number of fish remaining in the troughs at each initial density, *j*, at consecutive sampling time intervals, t_i and t_{i+1} (days). Two prominent linear trends in population decrease following the initiation of aggression were suggested (see Results, Fig. 2A). We used a series of linear regression analyses to determine the time corresponding to the minimum in the two regression lines' pooled residual sum-of-squares, i.e. the time when the delineation between the two trends in population decrease was strongest. Period 3 began at this time and continued until emigration ceased. Period 4 extended to the end of the experiment.

Wet weight (WW) and dry weight (oven-drying at 75°C for 24 h) of each fish were measured to the nearest milligram. In all, 348 brown trout were dissected for sagittal otoliths (large trap-catches during the first 2 d of the experiment were subsampled by use of a random numbers table and about 40%, or at least 10 individuals, were selected from each trough on each sampling occasion). Once removed, the otoliths were rinsed with deionized water, air-dried, and mounted on standard glass microscope slides with thermoplastic cement with the sulcal surface of the otolith facing upward. Some otoliths were ground with fine carborundum paper (1200 grain) and polished with 0.3-µm aluminum micropolish to enhance the visibility of microstructures.

A compound light microscope with an optical tracing tube allowed a simultaneous view of the microscopic field and a digitizing tablet. Otolith measurements were made by use of the digitizing tablet and the data were automatically recorded on a microcomputer. A distinct check mark was formed in the otoliths when water temperature was increased just before the start of the experiment (Mosegaard et al. 1987). This mark was used to measure total otolith length at the start of the experiment, i.e. emergence (L1, measured along the longest axis; Fig. 1). Total otolith length was also measured at the time the fish was removed from the experiment (L2; Fig. 1). In addition, a computerized image-digitizing system measured 60 evenly distributed radii (i.e. at every sixth degree on a 360° system) from the posterior primordium of the largest nucleus to the outer edge of the otolith (Fig. 1). Measurements began at the rostral peak (0°) and continued sequentially around the otolith, beginning with the dorsal edge. The roughly triangular area between sequential radii was also calculated and summed to approximate the total cross-sectional area of the proximal side of the sagitta.

These data were used for least squares linear regression analyses (SAS Institute Inc. 1985) with fry dry weight to investigate the relationship between otolith size indices and fry weight at emergence. All possible combinations of natural logarithm (ln) transformed and nontransformed data for both the dependent (fry dry weight) and independent variables (sagittal radii and total area) were used. Dry weights were considered to be the most reliable index of fry size at emergence and were used in preference to wet weights and total lengths. Small amounts of adsorbed water may cause relatively large errors in wet weight determinations of fry, and variation in fry lengths at emergence is minimal making it difficult to accurately measure size differences between individuals (R. Titus and H. Mosegaard, pers. obs.).

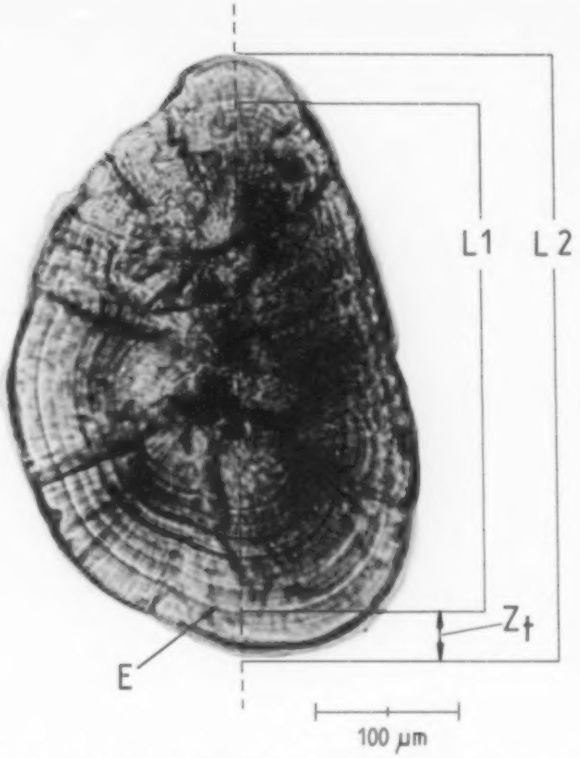

FIG. 1. Cross-sectional view of a sagittal otolith from a migratory brown trout fry. The letter E indicates the "emergence" check mark formed at the start of the experiment. L1 and L2 are total otolith length at emergence and at time of removal from the experiment, respectively. Z_t is total otolith incremental growth formed between emergence and time of removal from the experiment.

The image-digitizing system was also used to make automatic identifications and measurements of daily growth increments (rings) in otoliths, and manual readings of total incremental growth zones (Z_t), which were measured along the posterior portion of the longitudinal axis of the sagitta (Fig. 1). Temperature-independent, initial otolith growth rates (OG, micrometres per degree-day (DD)) (Mosegaard and Titus 1987) were determined by

$$\mathrm{OG} = \Delta\mathrm{OR} \cdot (\Delta\mathrm{DD} - a)^{-1}$$

where ΔOR is the width of the selected growth zone, ΔDD is the corresponding number of degree-days, and *a* is the *y*-intercept determined from linear regression analysis of degree-day development (*y*) on mean Z_t (*x*) in otoliths from downstream-moving fry 3–6 d after emergence. For fry remaining in the troughs beyond 6 d, OG was calculated using the width of the

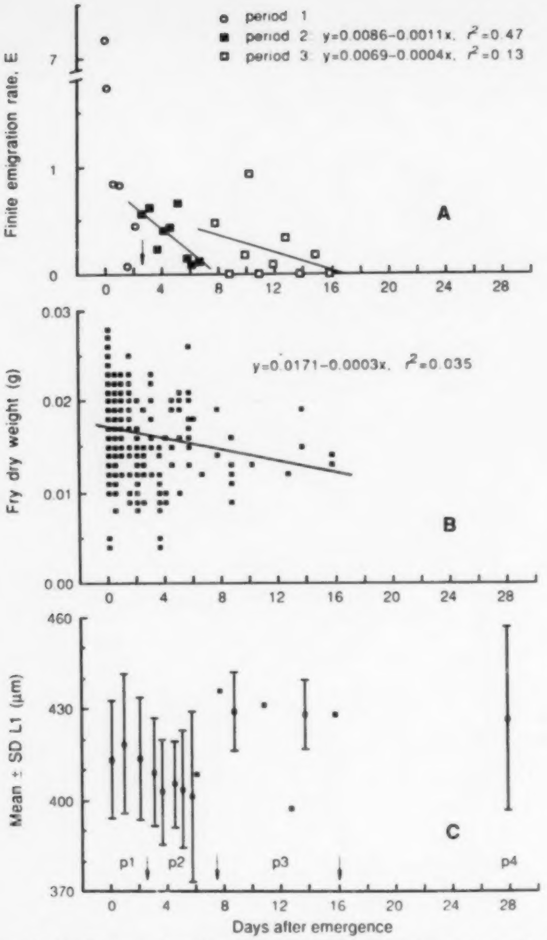

FIG. 2. (A) Finite emigration rates (E) of migratory brown trout fry in the artificial stream sections over time. The arrow indicates the time when aggressive interactions between fry were first observed. (B) Dry weight of trap-captured fry over time. (C) Total otolith length at emergence (L1) for each group of trap-captured fry over time (days 0–16). Mean L1 shown at day 28 is that of fry which remained in the troughs through period 4. The arrows indicate the division times between periods in population regulation.

incremental growth zone formed during the first 4 d after emergence. In this manner, initial otolith growth rates could be compared directly between fry groups.

Results

Periods of Population Regulation

Movement from the emergence baskets and downstream dispersal of fry began almost immediately following the start of the experiment (Fig. 2A). Aggressive interactions between individuals were first observed 2.5 d after emergence, thus marking the end of the first period of population regulation. Behaviors observed were charging, nipping, chasing, displaying, and fleeing as described by Stringer and Hoar (1955), Kalleberg (1958), and Keenleyside and Yamamoto (1962).

The minimum of pooled residual sum-of-squares for the two prominent linear trends in population decrease following the initiation of aggression occurred at 7.5 d, thus making the division between periods 2 and 3 (Fig. 2A). Emigration ceased after 14 and 16 d when the original populations of 68 and 136 alevins·m^{-2} had been reduced by 98 and 97%, respectively. Thus, period 3 ended at day 16 after emergence (Fig. 2A). Final densities (mean ± SD) in period 4 were 3.8 ± 1.3 and 1.1 ± 0 fry·m^{-2} in troughs with initial densities of 136 and 68 alevins·m^{-2}, respectively. These mean final densities were significantly different (p = 0.05, significance test for two Poisson samples) (Campbell 1974).

Fry Growth

Downstream-moving fry had decreasing weights following emergence, as based on the dry weights of trap-captured fry over time (Fig. 2B). This decreasing trend was significant (t-test of $b=0$, $p<0.001$). Among fry which remained in the troughs, final mean total lengths were 43.3 ± 3.1 and 43.1 ± 5.3 mm at initial densities of 68 and 136 alevins·m^{-2}, respectively. Corresponding mean final dry weights were 0.075 ± 0.025 and 0.080 ± 0.047 g. There was no significant difference in mean final total lengths and dry weights between initial densities (t-test, $p>0.50$).

Otolith Size at Emergence in Relation to Fry Size and Emigration over Time

Simple linear regression analysis poorly predicted fry dry weight as a function of total otolith length at emergence (r^2 = 0.06, n = 73). Low correlations were also found when the independent variable was each of 60 sagittal radii and total sagittal cross-sectional area ($r^2 \leq 0.09$). No major improvement was achieved when several regressors were used in multiple regression analyses ($R^2 \leq 0.18$). Therefore, reliable back-calculation of fry size at emergence from otolith size was not possible, and we were unable to investigate a potential size-selection mechanism among postemergent fry using this method.

However, a clear-cut selection pattern on total otolith length at emergence (L1) was suggested when mean L1 of fry on each sampling occasion was plotted against time (Fig. 2C). Fry which moved downstream during period 1 had otoliths of intermediate sizes, period 2 emigrants had the smallest otoliths, and period 3 emigrants and fry remaining in the troughs through period 4 had the largest mean otolith sizes at emergence.

Mean L1 in each of periods 3 and 4 was significantly greater than that in period 2 (t-test, $p<0.05$), but not period 1 ($p>0.05$). There was also no significant difference in mean L1 between periods 1 and 2 ($p>0.05$) or between periods 3 and 4 ($p>0.50$). However, the variation in L1 was significantly greater in period 4 than in period 3 (F-test for equality of variances, $p<0.05$).

These results suggest that there was no selection pressure, as reflected in otolith size at emergence, on emigrating fry during period 1, prior to the development of territorial behavior. However, as fry began to compete for territories during period 2, there was a strong selection against fry with small otoliths at emergence and these fry were forced to emigrate from the troughs. For fry remaining in the troughs in periods 3 and 4, selection clearly favored individuals with larger otoliths at emergence; this was especially apparent among fry which maintained territories through the end of the experiment.

Because of the very low correlation between otolith size and fish size at emergence (regression equation: $L1 = 386.93 + 1336.3 \cdot DW$; $r^2 = 0.06$, $p = 0.033$, $n = 73$, mean $L1 = 411.64$ µm, $S_{y \cdot x} = 21.51$), we concluded that selection for fish size alone could not account for the observed clear-cut pattern of otolith size selection (Fig. 2C). We tested this conclusion by determining the maximum effect a selection on fish size could have on the observed length frequencies of otoliths at emergence. The observed low mean L1 in period 2 fry, and the high mean L1 in period 3 fry, should therefore be explained by fry with low and high dry weights at emergence, respectively. Following this argument, fry which emigrated into the traps during period 2 should have exhibited positive growth to attain the observed dry weight at capture, while fry which emigrated into the traps during period 3 should have had negative growth.

Maximum mean specific growth rate of migratory brown trout fry is about 3.5% (wet weight) per day during the first month after emergence (Elliott 1984b). This growth rate was used to back-calculate dry weights at emergence for fry which emigrated from the troughs during period 2. Maximum starvation rate was estimated as 0.7 of maximum growth rate (Elliott 1982), i.e. 2.5% (wet weight) starvation per day. This starvation rate was used to back-calculate emergence weights of period 3 fry. From the back-calculated emergence dry weights of 37 and 10 fry from periods 2 and 3, respectively, total otolith length at emergence could be estimated and compared with observed values.

Estimated emergence dry weights (EDW) were used in the regression equation above with the stated error term ($S_{y \cdot x} = 21.51$) to produce randomized estimated total otolith lengths at emergence (EL1):

$$EL1 = 386.93 + 1336.3 \cdot EDW + RANNOR\ (21.51)$$

where RANNOR (SD) is a function which produces random, normally distributed numbers with a mean equal to zero and standard deviation equal to, in this instance, 21.51 (SAS Institute Inc. 1985). Comparisons were made with paired *t*-tests between observed (L1) and estimated (EL1) total otolith lengths at emergence for each period (2 and 3). For these comparisons, differences were calculated between each observed otolith length (L1) and 100 randomized estimated values (EL1). Based on these differences, a population mean ($EL1_{mean}$) and standard deviation ($EL1_{SD}$) were calculated for both periods and used to estimate the one-tailed probability of a zero mean, with df = number of emigrants per period − 1, i.e. $p\ (EL1_{mean} \cdot (\sqrt{n} \cdot EL1_{SD})^{-1} > t_{df})$.

The combined probability of attaining the observed mean L1 in periods 2 and 3, under the growth and starvation conditions specified above, was calculated as the product of the individual periods' probability, as shown in the table below (paired *t*-test statistics on the differences between individual observed (L1) and randomized estimated (EL1) total otolith lengths at emergence):

Period	Mean difference	SD	df	*t*	*p*(*t*)
2	1.185	29.17	61	0.320	0.35
3	−15.49	25.43	18	−2.655	× 0.007
					0.0025

With a combined probability of 0.0025, the observed pattern of otolith size selection was not likely the result of a selection for fry dry weight at emergence, even when extreme growth/starvation conditions were assigned to different parts of the population. In fact, quite the opposite conditions were actually indicated because qualitative features of otolith microstructures suggested that period 2 fry were starved while period 3 fry grew initially and later were starved (see Qualitative Features of Otolith Microstructures).

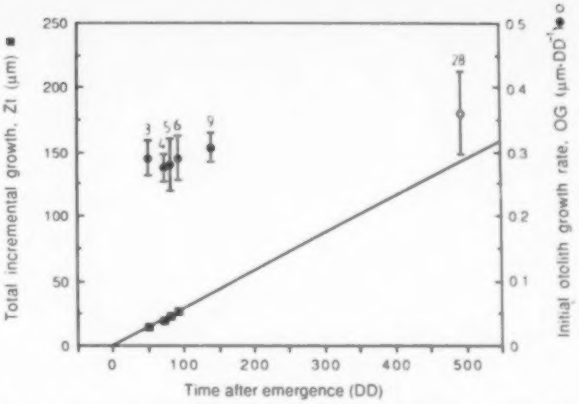

FIG. 3. Initial otolith growth rates (OG, mean ± SD) of downstream-moving fry during periods 2 and 3 (solid circles) and fry which remained in the troughs through period 4 (open circle). Time after emergence is expressed in degree-days (DD). Linear regression analysis of degree-day development on mean total incremental otolith growth for downstream-moving fry from period 2 (solid squares; axes reversed to facilitate graphic presentation) resulted in an *y*-intercept of 1.6 DD with which to calculate temperature-independent, initial otolith growth rates ($b = 3.44$, $r^2 = 0.99$, $p = 0.006$).

Initial Otolith Growth Rates

Initial otolith growth rates were compared between downstream-moving fry in periods 2 and 3 and fry which remained in the troughs through period 4 (Fig. 3). Initial otolith growth rates were significantly higher in fry which remained in period 4 than in both groups of downstream movers (Table 1). There were no significant differences in growth rates between groups of downstream movers.

Final Otolith Size – Fry Size Relationships

Among fry which remained in the troughs through period 4, final fish size (dry weight) was well predicted by final otolith length (L2; $r^2 = 0.83$, $p = 0.0001$). Final dry weight of period 4 fry was also analyzed in relation to otolith length at emergence (L1) and trough (1–6), using the interaction term between the latter two variables in an ANCOVA to take into account possible prey differences between troughs. The L1–trough interaction was highly significant ($p = 0.003$), and this alone explained 93% of the variation in final fry dry weight, with all partial regression coefficients being positive and similar (0.00051–0.00075). In addition, when ascending trough number (1–6) was used as a continuous dummy variable and an index of potentially decreasing prey quality through the experimental system, a significant linear relationship was found between final fry dry weight and the interaction term of L1 and trough number ($r^2 = 0.46$, $p = 0.015$). Thus, ascending trough number explained part of the variation in final fry dry weight.

TABLE 1. Analysis of variance of temperature-independent, initial otolith growth rates (OG) for downstream-moving fry in periods 2 and 3 and for fry which remained in the troughs through period 4.

OG in periods	Source of variation	df	SS	$p > F$
2 versus 3	Between periods	1	0.0003	0.57
	Within periods	34	0.034	
	Total	35	0.035	
2 versus 4	Between groups	1	0.031	0.0001
	Within groups	41	0.057	
	Total	42	0.088	
2 + 3 versus 4	Between groups	1	0.031	0.0001
	Within groups	45	0.058	
	Total	46	0.089	
3 versus 4	Between groups	1	0.012	0.02
	Within groups	14	0.025	
	Total	15	0.038	

Qualitative Features of Otolith Microstructures

Differences between downstream-moving and remaining fry were also reflected in qualitative otolith microstructure patterns. Otoliths from fry which moved downstream during period 2 had transparent microstructures and almost never had discernible daily rings (Fig. 4A). In otoliths from period 3 emigrants (Fig. 4B), most individuals had initially discernible rings, but the structures near the edge of the otolith were more transparent, with no clear ring formation except for an occasional check mark similar in appearance to the "emergence" check. Otoliths from period 4 fry all exhibited clear daily increments during the first 8 d (Fig. 4C); microstructures formed thereafter were more transparent with a faded appearance of daily rings, probably in association with water temperatures rising above 17°C (Mosegaard and Titus 1987).

Discussion

An uncoupling between otolith and somatic growth rates in salmonid fry as an effect of differential temperature response has been demonstrated previously (Mosegaard and Titus 1987; Mosegaard et al. 1988). Available evidence was used to develop an alternative hypothesis to explain otolith growth rates whereby otolith microstructure formation is coupled to fish metabolism (Mosegaard 1986; Mosegaard et al. 1988). Fish functioning at a high metabolic rate are predicted to have a corresponding high rate of otolith formation. Thus, otolith size at emergence will reflect the standard metabolic level of the individual fish through the egg and alevin stages when nutrition is constant and exercise is minimal, although not necessarily the fish body size at emergence, as evidenced by the weak relationship between otolith size and fish size variables in this study.

Natural selection is predicted to favor genotypes which are metabolically efficient and able to increase their metabolic scope at lower power outputs (Priede 1985). The observed selection for individuals with greater mean otolith size at emergence suggests that these fish had a higher metabolic scope. By having a high metabolic turnover rate, energy may be allocated to both somatic growth and territorial defense at a higher rate, assuming energy intake can also be high. These individuals may be regarded as having a greater metabolic, and hence growth, potential from emergence, which later in the growth history is reflected in larger body size by virtue of their growth advantage (i.e. high somatic growth rate). Further, greater body size generally confers dominance in the social hierarchy of stream salmonid fry populations (e.g. Chapman 1962; Mason and Chapman 1965; Fausch 1984). Indeed, selection for fry with larger otoliths at emergence, despite weak otolith size – fish size relationships at emergence, has been demonstrated elsewhere (West and Larkin 1987; but see also Mosegaard 1990).

We propose that fry selection at emergence was not based on fish size, but instead on the metabolic performance of the fish as reflected in relative otolith size. This process is therefore termed selection for greater growth potential.

The significantly higher initial otolith growth rates (OG) of fry which remained in the troughs through period 4 were congruous with the finding of larger otolith size at emergence (L1) for that group. The results of otolith microstructure analysis among period 4 fry also showed that the weak correlation between fry size and otolith size at emergence was replaced by a very strong relationship after 1 mo (between final DW and L2) and that otolith size at emergence strongly influenced subsequent fry growth (as final DW) taking potential prey differences between troughs into consideration. Part of the variation in final fry dry weight may have been due to decreasing prey quality through the experimental system. If these results are viewed in light of the hypothesis of metabolism-controlled otolith growth (Mosegaard et al. 1988), then differences in fry metabolism at emergence not only act upon selection following emergence, but also influence subsequent growth rates of individual fry which establish territories. These findings support further that a selection for greater growth potential did indeed occur.

The large amount of variation in otolith size at emergence among remaining fry (Fig. 2C) may be explained by the presence of both dominant and subordinate fish in the final populations. Theoretically, greater growth potential (GGP) individuals may be equated with dominants, while subordinates (presumably represented by smaller otolith size at emergence) may be regarded as having lesser growth potential (LGP). Indeed, LGP individuals would function well as subordinates at suboptimal feeding positions because their lower metabolic scope (and hence lower growth rates) would require less energy intake. As Metcalfe (1986) suggested, the optimum growth strategy for subordinates may not be one of attempting to maximize food intake, but one of minimizing energy expenditure by assuming sheltered (poor) feeding positions away from faster currents where dominants occur. That is, the fish will continue to grow (albeit slowly) and survive despite a low feeding level. It could be further argued that under conditions of overall low prey availability, selection could be reversed to favor individuals with lower metabolic scope (smaller mean otolith sizes at emergence) and lower energy demands for growth and survival (i.e. selection for lesser growth potential).

The final group of emigrants (period 3 fry) had nearly equal mean otolith sizes at emergence (L1) as fry which remained in period 4. Some studies on the behavioral ecology of territorial salmonid fry have reported that aggression rates among fry are greatest between fish of nearly equal length (e.g. Chapman 1962; Wankowski and Thorpe 1979; but see Stringer and Hoar 1955; Grant et al. 1989). Period 3 fry were presumably able to remain in the troughs longer than period 2 emigrants because

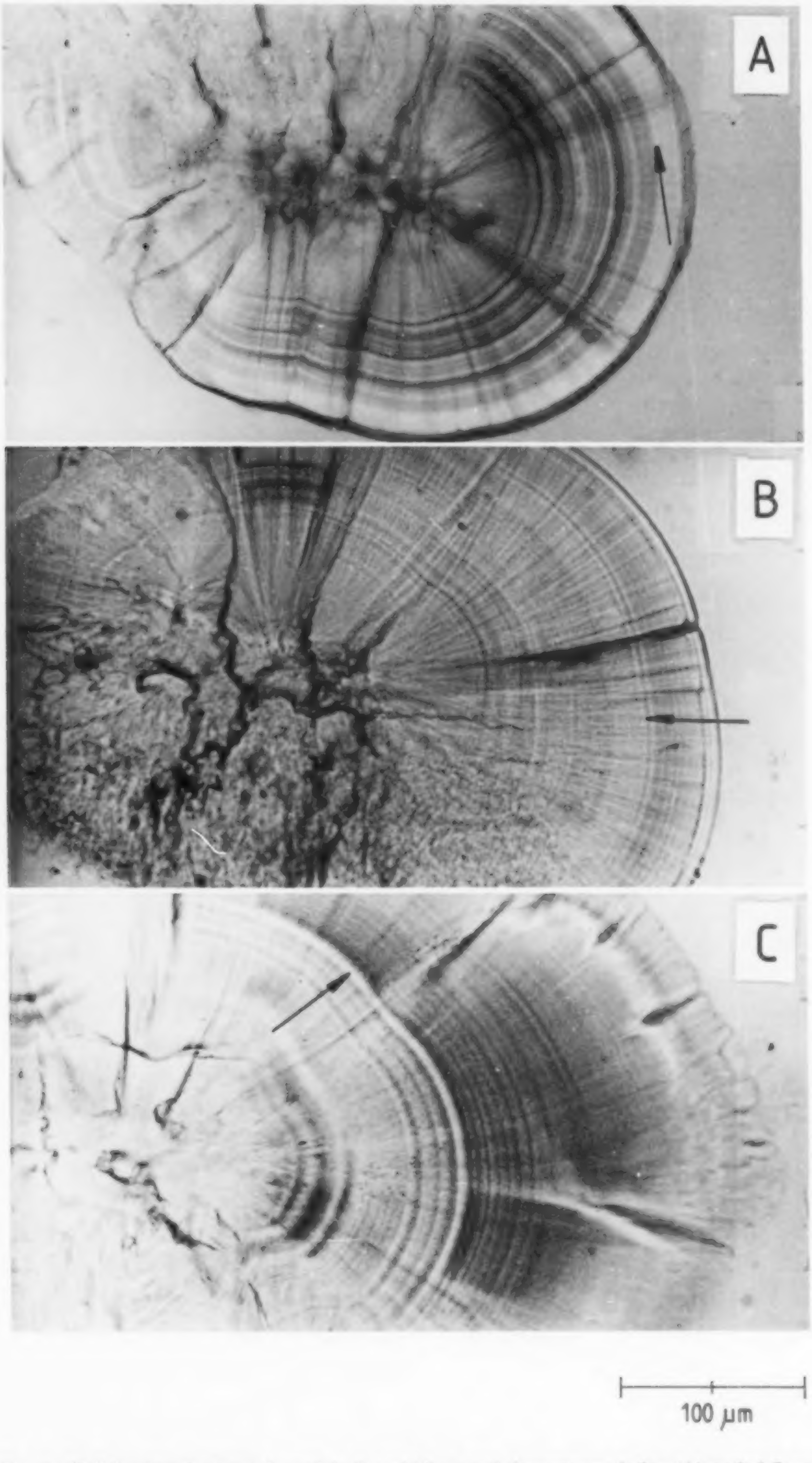

FIG. 4. Otolith microstructures formed in fry which moved downstream during (A) period 2 and (B) period 3 and (C) in fry which remained in the troughs through period 4. The arrows indicate the "emergence" check mark formed at the start of the experiment.

of their metabolic advantage, as reflected in relative L1 (see argument above). However, they may have also been met with intense competition for feeding positions with period 4 fry of similar metabolic ability, i.e. similar L1, and were eventually forced to move downstream due to starvation (Fig. 2B). Indeed, the fry in these two groups may have been similar in body size because by the end of the experiment, final fish size was strongly correlated with final otolith size. Further, during period 3, fry with small otoliths at emergence had either already emigrated (in periods 1 or 2) or were among those that had presumably established themselves at suboptimal feeding positions as LGP subordinates through period 4. This may explain the small variation in otolith size at emergence among period 3 emigrants. Thus, period 3 may have been the final major competition period among GGP dominants.

The differences in otolith microstructure appearance between downstream-moving fry and fry which remained in the troughs may reflect differences in feeding status (Rice et al. 1985; Mosegaard 1986). Transparent zones near the otolith periphery in the downstream movers indicated that they experienced a period of starvation before they migrated or were forced out of the stream sections. That downstream movers did not feed was also supported by the gradual decline in dry weight of these fish (Fig. 2B).

In a natural stream, the fate of downstream migrants would not be certain; some would probably establish feeding territories downstream, while others would continue to starve and die. Elliott (1984a, 1986) regarded migratory brown trout fry that migrated downstream out of the nursery area as losses from the population not due to emigration but to actual death within the stream. He based this assumption on the fact that downstream migrants had not fed, lost weight, and were moribund due to malnutrition. These "doomed" downstream migrants accounted for about 81% of emerging fry (Elliott 1986). The fate of a given individual moving downstream would depend on both intrinsic factors such as its size, condition, and individual metabolic and behavioral characteristics as well as extrinsic factors such as the quality and availability of feeding positions, the probability of encountering fry which have already established downstream territories, and predation risk. Theoretically, the fry's chance for survival will continually decrease the further downstream it must move in order to find a free territory or take over another and begin feeding.

Selection for fry with larger otoliths at emergence, despite a weak correlation between otolith size and fry size at emergence, suggests that otolith size at emergence reflects a determinant fry characteristic besides its size. This was also Mosegaard's (1990) conclusion in a reworking of similar results with sockeye salmon (*Oncorhynchus nerka*) from West and Larkin (1987). We propose that otolith size at emergence instead reflects the metabolic and hence growth potential of the individual fry. Future work should endeavor to explain further the determinants of otolith formation rates and qualities. With this knowledge, otolith microstructure analysis may be applied with even greater confidence to reveal characteristics of individual fry which may be influenced by natural selection.

Acknowledgments

We thank Nils G. Steffner, Bjarne Ragnarsson, and the staff of the Fisheries Research Station at Älvkarleby who provided experimental facilities and lodging, as well as assisted with data collection. Magnus Appelberg, Erik Degerman, and Birger Pejler provided comments which improved the manuscript. This work was financed by grants from the Research Council of the National Swedish Board of Fisheries, the Swedish Council for Forestry and Agricultural Research, and the Institute of Limnology, Uppsala University.

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Effects of a Pulp Mill Effluent on the Population Dynamics of Perch, *Perca fluviatilis*

Peter Karås, Erik Neuman, and Olof Sandström

National Swedish Environmental Protection Agency, Coastal Water Laboratory, P.O. Box 584, S-740 71 Öregrund, Sweden

Karås, P., E. Neuman, and O. Sandström. 1991. Effects of a pulp mill effluent on the population dynamics of perch, *Perca fluviatilis*. Can. J. Fish. Aquat. Sci. 48: 28–34.

The dynamics of a Baltic perch (*Perca fluviatilis*) population were studied in a bleached pulp mill effluent area on the Swedish coast. Recruitment was seriously affected, as larval and fry abundances were very low. Studies of spawning on artificial substrates demonstrated that sufficient numbers of eggs were deposited even in the most effluent-exposed parts of the study area. No increased egg mortality could be noted, but at the stage close to hatching about 10% of the embryos were observed to be malformed, generally having sharp bends in the posterior part of the spinal cord. Exposed embryos were generally smaller than reference fishes, both when length and weight data were compared. Larval samplings provided evidence for high mortality at or very close to hatching. Food and feeding conditions appeared to be of little importance for mortality. Two mortality causes were suggested: chronic failure of parental reproductive systems and/or acute toxicity to embryo or early larvae.

On a étudié la dynamique d'une population de perche (*Perca fluviatilis*) dans une zone d'effluents d'usine de pâte blanchie sur la côte suédoise. Le recrutement était gravement touché, car l'abondance des larves et des alevins était très faible. Des études du frai sur des substrats artificiels ont montré que les oeufs étaient déposés en nombre suffisant, même dans les parties de la zone étudiée qui étaient le plus exposées aux effluents. On n'a remarqué aucune augmentation de la mortalité des oeufs, quoiqu'une fois le stade d'éclosion presque atteint, on a noté que 10% des embryons étaient difformes : en général, la partie postérieure de la moelle épinière était coudée. La taille des embryons exposés était généralement inférieure à celle des poissons de référence, tant en longueur qu'en poids. Les échantillons larvaires ont montré une mortalité élevée à la période d'éclosion ou à la période précédant immédiatement l'éclosion. La nature des aliments et les conditions d'alimentation ne semblaient pas influer beaucoup sur la mortalité. On soupçonne deux causes de mortalité : l'insuffisance chronique du système reproducteur chez les parents et la toxicité aiguë chez les larves précoces ou les embryons.

Received November 14, 1989
Accepted July 24, 1990
(JA360)

Reçu le 14 novembre 1989
Accepté le 24 juillet 1990

Pulp mill effluent contains a mixture of environmentally active substances. Dissolved nutrients and dissolved and particulate organic matter cause eutrophication. The release of resin acids and the organochlorine wastes from mills producing bleached pulp can add toxic stress. Beginning in 1982, studies on the biological effects of pulp production were made in an effluent area on the Baltic coast, chosen as representative of Swedish bleached sulphate pulp mills. A number of reports have been published, covering chemistry, sediments, benthic vegetation and fauna, and finally fish (review by Södergren 1989).

In the efforts to reveal the principles governing fish responses, a relatively sedentary species, the perch (*Perca fluviatilis*), was chosen for combined physiological and ecological studies. A diverse response pattern was observed in the exposed population. Fish appeared to be in poor health (Andersson et al. 1988). Studies of reproduction, growth, and survival revealed that the risk of death was increased and that the production of sex cells was lowered (Sandström and Thoresson 1988; Sandström et al. 1988). Growth rate, however, was increased in exposed fish (Sandström et al. 1988). The number of fry produced was low, and the adult population was drastically reduced in the vicinity of the mill (Neuman and Karås 1988). Perch was not the only species affected, and recruitment failure seemed to be general, as larvae and fry of other common Baltic fishes were nearly absent from the most exposed areas (Neuman and Karås 1988).

As recruitment damage is a serious consequence of pollution to a fish population, a sampling program was designed covering the distribution of the adult population in relation to the effluent, spawning observations, egg survival, embryo qualities, larval abundances, and larval feeding. The main objective was to elucidate whether recruitment was affected by low spawning intensities, increased egg or larval mortality due to chronic damage in the parents, acute effects on the spawn by the effluent, or low food availability for the larvae.

Effluent Area

The effluent area studied is situated in a shallow bay on the southwest coast of the Bothnian Sea, northern Baltic (Fig. 1). Salinity is low but stable, at about 4‰, as there are no major freshwater inputs. Water exchange is comparatively slow in the bay and in the nearby reference area, as there are no rivers and as tidal currents are insignificant in the Baltic. Apart from the pulp mill, there are no other local pollution sources. The topography allowed selecting sampling stations of similar exposure to the open sea, similar temperature conditions, etc., at increasing distances from the effluent discharge.

Bleached sulphate pulp is produced at the mill. The yearly production during 1982–88 and the effluent characteristics are

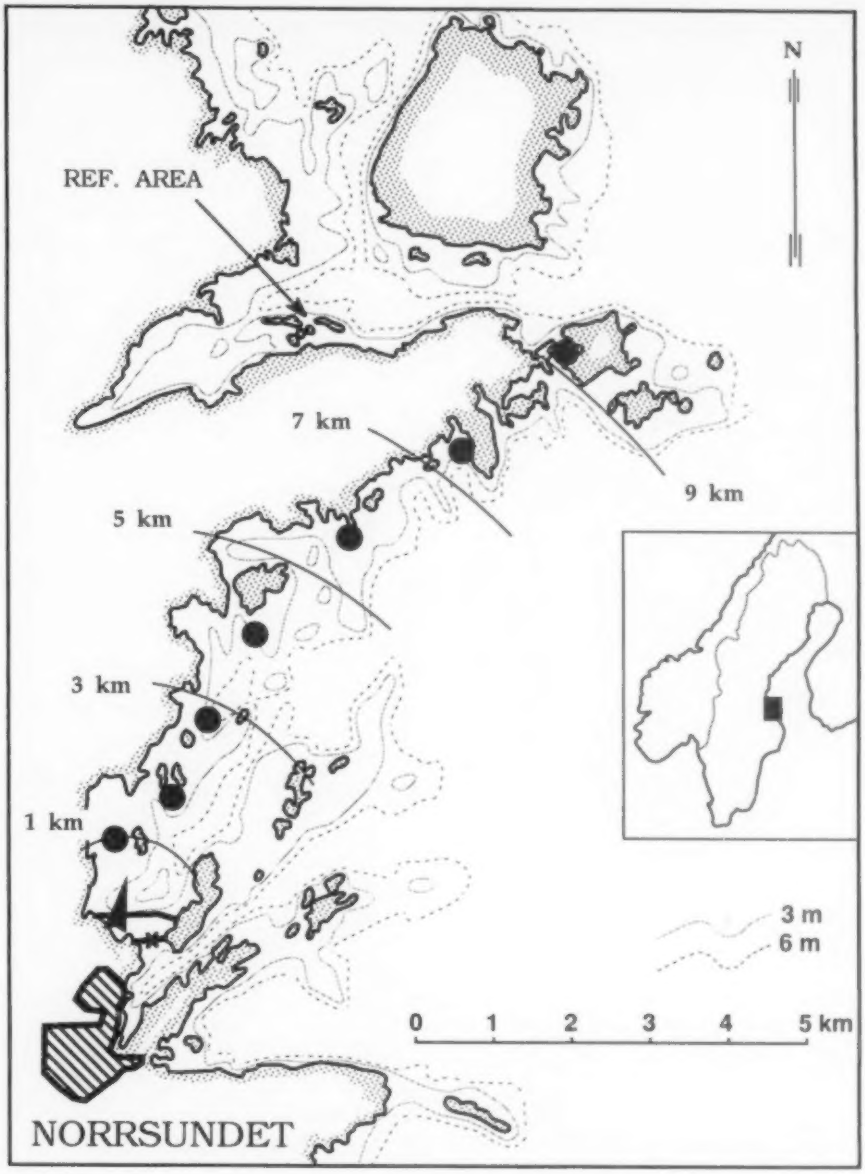

FIG. 1. Effluent area. ● = sampling station.

TABLE 1. Effluent characteristics and production data from Norrsundet pulp mill during 1982–88. (Susp. = suspended solids; COD = chemical oxygen demand; Cl = elementary chlorine used in bleaching; data supplied by Norrsundets Bruk AB).

	Production (1000 t)						
Year	Total	Bleached	Susp. (kg/24h)	COD (t/24 h)	Cl (t/yr)	Total P (kg/24 h)	Total N (kg/24 h)
1982	133	121	2240	53	10 649	30	150
1983	203	203	2230	69	11 931		
1984	234	234	1780	51	8 731	37	80
1985	222	222	1800	36	6 774	26	105
1986	228	228	2000	40	6 911	28	104
1987	240	240	1000	36	6 894	37	
1988	244	244	1300	37	6 215	36	110

presented in Table 1. Bleaching starts with an oxygen delignification. The pulp is distributed to two separate bleaching lines with the sequences O(C+D15%) (EO)D and O(C+D15%) (EO)DED. The effluent characteristics were fairly representative of Swedish bleached sulphate pulp mills in the early 1980's. Although pulp production increased, modifications during 1982–83 and further technical improvements led to decreased total discharges of oxygen-consuming substances (COD) and organochlorine compounds resulting in a considerably lower environmental load during 1985–88. The mill effluents were diluted approximately 60 times at the discharge point as they were mixed with seawater pumped from a nearby bay.

Material and Methods

The main studies were made in 1987 and 1988, but additional information was used from investigations during 1982–85 (Neuman and Karås 1988; Sandström et al. 1988).

Population Studies

Studies on the distributions of stocks were made in August 1982, 1984, 1985, 1987, and 1988 using gill nets set at the bottom at distances of approximately 1, 2, 3, 4, 6, 7, and 9 km from the mill (Fig. 1). The water depth at the sampling stations was about 4 m, thus covering the main habitats for perch and other freshwater coastal species in the Baltic. August samplings do not represent the distribution of the spawning population in spring (May–June) but provide reasonable estimates of relative abundances and give reliable information on stock variations. A description of sampling techniques, etc., is given in Neuman and Karås (1988).

When analysing catch data, the between-year differences in mean catch per effort depending upon, for example, temperature variations were eliminated by a normalization technique. The mean catch per effort calculated for all stations in a single year was set as 1, and the catches at different stations were related to this standard. Consequently, when the mean catch at a station was twice as large as the mean for all stations, the normalized catch was expressed as 2 in the graphic presentation. The Kruskal–Wallis test (Kruskal and Wallis 1952) was used for the comparisons of stations, accompanied by a nonparametric method analogous with Neuman–Keuls' test (Zar 1974). Differences were considered significant at the 5% level.

Spawning Studies

During spawning, female perch attach a single continuous string of roe at depths normally less than 2 m on vegetation, tree branches, etc. To estimate spawning intensities, approximately 2-m-long immersed spruce tops were used as artificial substrates. Introductory studies during earlier spawning seasons had shown that these substrates were readily accepted by the females. Groups of six spruce tops submerged at approximately 1.5 m depth were placed 1, 2, 3, and 4 km from the mill and in the reference area (Fig. 1) in early spring 1987. Additional spruce tops were placed close to the sampling stations in identical conditions as substrates for the incubation of collected roe strings. From the beginning to the end of the spawning season, the attached roe strings were counted about weekly and transferred to neighbouring substrates for continued embryonic development. Close to hatching, samples were taken to study egg mortality, embryonic quality, etc.

Egg and Embryo Studies

The roe samples were preserved in formaldehyde and studied in the laboratory. Embryo mortality was estimated on subsamples of 100–150 eggs from each of the collected roe strings. Ten roe strings, in the stage close to hatching, collected 1 km from the mill and 11 strings from the reference area were used for the further comparisons. Two hundred embryos per string were checked for morphological deformations. Total lengths were measured on 25 embryos per string, total weight on 100 per string, and weight without yolk sac on 100 per string. The weight measurements were made on pooled samples.

Larval Sampling and Studies of Larval Feeding

A twin coupled Bongo net (mesh size 0.3 mm) equipped with a flowmeter was towed 0.5–1 m beneath the surface in daytime larval samplings in areas with a water depth of about 2 m. The samplings were made 1, 3, and 6 km from the mill and in the reference area, six times during the period from the beginning of June to the end of July 1987. Five trawlings per area, generally filtering 50–100 m^3, were made at each sampling date. The Bongo sampler has been tested against other samplers and found to provide comparatively accurate estimates of larval abundances (Bjorke et al. 1974). However, the efficiency decreases at larger fish sizes, and the technique is only suitable for perch larvae shorter than 18 mm (Karås 1987). The larval samples were preserved in formaldehyde. The numbers of larvae were counted in the laboratory and total lengths were measured to the nearest millimetre.

Larvae collected in the Bongo nets were used in a food consumption study. During early larval development, sufficient numbers of larvae were only available from samplings made 3 km from the mill and from the reference area. Both yolk sac larvae and later stages were studied. The number of organisms of different taxa in the alimentary tract were counted under a light microscope. Comparisons were made with zooplankton samples, preserved in 70% ethylalcohol. The collections were made 1 and 3 km from the mill and in the reference area on July 8, when larval abundance was high. In each area, three 20-L water samples taken at a depth of about 1 m were pumped through a plankton net with a mesh width of 25 μm. With this mesh size, all relevant food items for perch larvae are retained in the samples. The samples were studied under a stereomicroscope. When possible, the analysis was performed at the species level. The genera *Asplanchna* and *Synchaeta*, however, are not easily separated in ethylalcohol-preserved samples. In the Baltic, *Synchaeta* spp. dominate but as *Asplanchna* spp. may occur in coastal areas, these two genera were treated collectively in the analysis.

Fry Abundances

Perch fry were sampled at the end of the 1984 growth season with an underwater detonation technique (Neuman and Karås 1988) enabling collections in all littoral habitats including rocky shores and dense vegetation. The relative abundance estimates provided have been shown to give reliable indications of recruitment (Karås 1987). Samplings were made on three different days in October in sheltered areas, less than 2 m deep, close to the gillnet stations. Six detonations were made per sampling station each day. Methods identical to those used in the studies on the adult population were employed in the statistical comparisons.

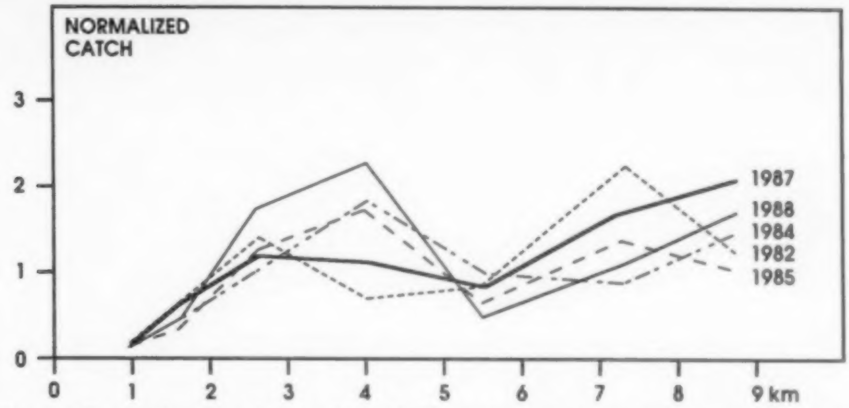

FIG. 2. Gillnet catches of perch during 1982–88. The catch at a single station is presented in relation to the yearly mean over all stations.

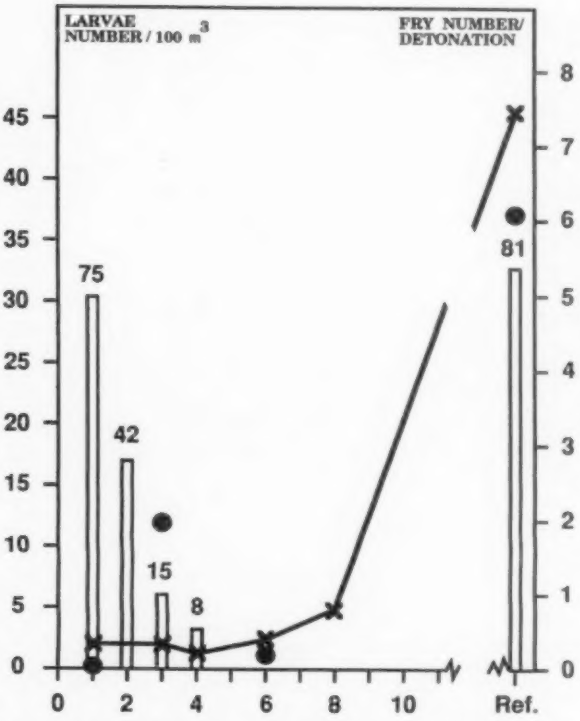

FIG. 3. Total number of roe strings collected from artificial substrates (columns), larval abundances (●) in Bongo net trawlings, and young-of-the-year caught by underwater detonations (×) at different distances from the mill.

Results

Population Distributions

The patterns of catch per effort of adult perch during the summer growth season, August, were remarkably stable between years, with significantly lower catches in the two sampling areas closest to the mill when compared with the reference area (Fig. 2). Although effluent conditions improved markedly during the sampling period, no clear effects could be seen on the catches.

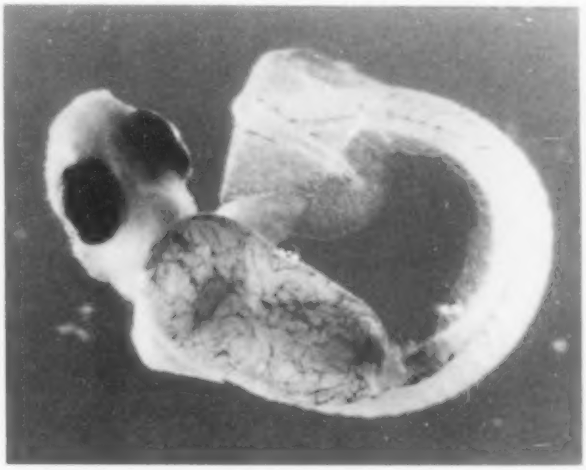

FIG. 4. Perch larva collected in the effluent area, 1 km from the mill, with a sharp bend on the posterior part of the spinal cord.

Spawning Intensities and Roe and Embryo Studies

In the effluent area, the total number of roe strings attached on the artificial substrates during the spawning season increased when approaching the mill (Fig. 3). Spawning seemed to be of similar intensity in the most exposed area 1 km from the mill and in the reference area.

Egg mortality was low and there were only small differences between sampling areas. Compared with 1.1% dead at the reference site, the frequencies of dead eggs in the samples from the effluent area were 1.9, 1.6, and 1.1%, respectively. Close to the mill, 10% of the exposed embryos were malformed, compared with about 1% in the reference samples. The most common deformation was a sharp bend in the posterior part of the body (Fig. 4), but deformations on the primordial fins also occurred. Exposed embryos were significantly shorter than the reference fishes (Student's t-test, $p < 0.05$, mean lengths 4.7 and 5.1 mm, respectively). Mean total dry weights (14.8 mg compared with 17.6 mg at the reference), mean dry weights without yolk sac (7.3 mg compared with 9.8 mg), and the calculated yolk sac mean weights (7.5 mg compared with the ref-

TABLE 2. Length distributions of perch larvae collected in the effluent area and in the reference area.

	Length group (mm)									
Area	5	6	7	8	9	10	11	12	13	14
1 km		2			1					3
3 km	7	38	14	4	6	6		3		1
6 km		3	4	1	1					
Ref.		98	209	124	100	42	11			

erence value 7.8 mg) also differed similarly, although not statistically significant at the 5% level.

Larval Abundances

As no perch larvae occurred in the first Bongo net samplings on June 2, 1987, and only very few when the study period ended on July 28, the hatching period was covered by the samplings in all studied areas. At the beginning of July, when peak abundances were reached, larvae of very different sizes occurred in the catches. For the comparisons, a relative estimate of larval recruitment was calculated by summing the total number of newly hatched (<9 mm) per 100 m^3 observed in the catches during the study period. Few perch larvae were caught in the most exposed area (Fig. 3). Hatching and larval survival occurred in the area 3 km from the mill, although the abundances were low. Further out in the receiving waters, 6 km from the mill, larvae were sparse. Compared with the exposed areas, larval recruitment was significantly (Student's t-test, $p < 0.05$) higher in the reference area.

The length distributions of larvae sampled 3 km from the mill and in the reference area, respectively (Table 2), showed that the larvae from both areas had survived the yolk sac stage, which normally ends at a length of 6–7 mm, and had begun external feeding. The comparatively lower number of older larvae in the effluent area indicates a higher mortality rate than in the reference population (Table 2).

Available Food, Consumption, and Food Selection

Potential food items for perch larvae, rotifers and copepod nauplii, occurred in all zooplankton samples (Table 3). Apart from the high rotifer abundances in the area 3 km from the mill, there were generally small differences between sampling areas.

As only few larvae were available from the sampling areas at 1 and 6 km from the mill, stomach analysis was conducted on larvae collected 3 km from the mill and in the reference area. Perch larvae began feeding at the yolk sac stage in both areas. The frequency of empty stomachs in post yolk sac larvae was somewhat higher in the exposed sample, but in larvae over 7 mm in length, no empty stomachs were found (Table 4). Prey selection may change quickly during the larval stages, and larger plankton animals, e.g. copepodits, were mainly consumed by larger larvae. Some differences were observed between exposed larvae and reference fishes. *Asplanchna* spp./*Synchaeta* spp. and rotifer eggs were predominantly eaten in the reference area, while copepod nauplii and copepodits were preferred in the exposed area. The mean number of food particles per stomach was higher in the reference area. Exposed larvae had consumed prey of larger size; calanoid and cyclopoid copepodits were eaten immediately after the yolk sac stage.

Surviving Fry in Autumn

In the study of perch fry in autumn 1984, relative abundance was generally very low at all sampling sites except in the most distant area visited, 9 km from the mill (Fig. 3). The differences were statistically significant. Results from seine net samplings made in 1984 and 1985 (Neuman and Karås 1988) demonstrated similarly that the abundance of perch young-of-the-year was very low in the effluent area.

Discussion

The observations on larval abundances and the occurrences of young-of-the-year in autumn (Fig. 3) demonstrated that the recruitment of perch was seriously impaired in a large part of the effluent area. Natural coastal areas of this type with closed topography and low mean water depths should, in contrast, be favourable for recruitment and should export fry and young fish to the surrounding waters (Karås 1989). In spite of the scarcity of young, adult fish were abundant except in the most exposed part of the effluent area (Fig. 2). Immigration from surrounding recruitment areas thus may occur, widening the ecologically affected area beyond that of immediate exposure. This hypoth-

TABLE 3. Abundances (number per 20 L) of potential prey items in zooplankton samples collected on July 8 at different distances from the pulp mill (m = mean; SD = standard deviation).

	1 km		3 km		Ref. area	
Species	*m*	SD	*m*	SD	*m*	SD
Rotatorians						
Asplanchna spp./*Synchaeta* spp.	510	221	3283	3694	623	108
Keratella spp.	21	9	2303	1721	1930	791
Kellicottia longisp.	10	10	0		0	
Cladocerans						
Bosmina coregoni	10	17	17	6	27	21
Podon spp.	20	10	150	66	7	12
Chydorus sphaericus	0		20	20	0	
Copepods						
Copepodits	20	10	76	64	27	31
Nauplii	683	303	450	20	410	96

TABLE 4. Stomach contents in perch larave collected 3 km from the mill and in the reference area. Larvae exceeding 11 mm in length were excluded from the analysis. Mean numbers (*m*) of different prey and their occurrence (percentage of total number of larvae) in different length classes are given.

		Yolk sac larvae		Length class (mm)					
				<7		7–9		9–11	
Area	Species	*m*	%	*m*	%	*m*	%	*m*	%
3 km	*Keratella* spp.			0.1	1				
	Asplanchna/*Synchaeta* spp.	1.7	61	1.9	40				
	Rotifer eggs			0.1	3				
	Calanoid copepodits			1.3	29	1.3	40	2.5	43
	Cyclopoid copepodits			0.1	3	1.6	47	1.8	31
	Nauplii			1.1	24	0.4	13	0.2	3
	Cladocerans							1.3	23
	Unspec. eggs	0.2	7						
	Number of larvae	10		15		9		6	
	Prey per stomach	2.8		4.6		3.3		5.8	
	% empty stomachs	78		25		0		0	
Ref.	*Asplanchna*/*Synchaeta* spp.	6.7	69	8.8	80	8.3	74	5.3	73
	Rotifer eggs	0.3	3	0.2	2	0.3	3	0.3	5
	Calanoid copepodits					0.6	5	0.5	8
	Cyclopoid copepodits					0.4	4	0.3	5
	Nauplii	2.0	21	1.8	16	1.6	15	0.6	8
	Cladocerans			0.1	1				
	Unspec. eggs	0.7	7	0.1	1				
	Number of larvae	3		49		78		64	
	Prey per stomach	9.7		11.0		11.2		7.2	
	% empty stomachs	90		11		0		0	

esis was supported by additional evidence from age distribution studies (Sandström and Thoresson 1988). Fish staying in the effluent area are known to die at a faster rate than normal (Sandström and Thoresson 1988), adding to the losses of recruits.

A primary question is whether low larval perch abundance is a consequence of low spawning intensities in the study area or an effect of toxic effluents. The spawning substrate studies demonstrated that the perch population tended to deposit their eggs in the inner parts of the bay and that the roe string densities were similar to those at the reference area. Although artificial substrates never can give fully quantitative estimates of spawning intensities, the results demonstrate that the recruitment deficit was not due to insufficient spawning.

We found no significant decrease in egg or embryo survival in roe strings deposited in any of the study areas. However, there were higher frequencies of damaged embryos and lower embryo sizes at high effluent exposures. Such impairments may be critical for survival, as the susceptibility to toxicants may be increased, and the larvae may not be able to hatch or swim properly. The smaller embryo sizes may have resulted from effluent-induced increases in metabolic rates. However, low embryo quality may also be of a more chronic origin related to previously demonstrated malfunctions in parental sex organs, affecting both sexes. The frequencies of potentially adult fishes observed with arrested gonad growth in autumn were high, up to 50%, and the remaining exposed developing adults were shown to have significantly smaller gonads than reference fishes (Sandström et al. 1988). A histological study of gonad preparations could not detect any general occurrence of damage to germ cells or reproductive tissues, and the conclusion was that the growth of the individual egg cell was slower in exposed females (Sandström et al. 1988).

Among the studied roe string samples, there was, however, also seemingly healthy material. Consequently, acute effects on larvae may occur. From laboratory experiments (review by McKim 1977), it is generally recognized that the larva is most sensitive to toxic action during the early yolk sac stage and the transition period between internal and external feeding. In our study, the main mortality must have occurred at or soon after hatching, coinciding with this low tolerance period, as practically no yolk sac larvae were collected in Bongo nets although the effort made was considerable. Strong currents, sweeping away the pelagic larvae, could provide an alternative explanation to their absence at certain stations. This is, however, not likely, as the water exchange is slow in this bay. Although the sampling stations had similar exposure to the open sea, small differences occurred and the area close to the mill was more protected from currents than more distant parts of the bay, including the reference area.

Mortality probably occurred near hatching before the larvae began feeding. Feeding or food availability may still be significant for survival in moderately affected parts of the receiving water area, i.e. 3 km from the mill, where larval mortality rates seemed to be higher than in the reference area. At the Baltic coast, perch larvae mainly prey upon the generally abundant rotatorians and their eggs, copepod nauplii, and early calanoid copepodits. Cladocerans generally are of minor importance (Karås 1987). The zooplankton samples demonstrated that suitable food items occurred during the period of maximal larval occurrence in all areas compared (Table 3) in abundances well above those considered necessary for successful growth and survival (Whiteside et al. 1985; Karås 1987). Still, there were deviations in food intake and food selection. In the exposed area 3 km from the mill, the frequency of empty stomachs was higher and the number of food items per specimen lower. Although the absence of perch larvae from the most exposed area evidently was not primarily related to feeding conditions, these differences suggest that food intake can be affected by pulp mill effluents.

Recruitment failure can fundamentally affect the population. In this study, a short and definite period was isolated during the early development, when mortality due to the exposure to mill effluents seemed to be high enough to significantly alter population dynamics. The ultimate cause of death was either chronic damage to parental sex organs, acute toxicity to larvae, or, most likely, a combination of both. Low spawning intensities and inadequate feeding conditions could not explain the low production of young fish.

Acknowledgements

We sincerely express our gratitude to Kjell Wilund, responsible for field samplings, Rose-Marie Svensson and Inger Abrahamsson for processing the laboratory materials, Bert Nyman for the preparation of text figures, and Lars Lindgren who made effluent data available to us. The research was financed by a grant from the National Swedish Environmental Protection Board, No. 5327088-0.

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A Test of the Relationship between Otolith Weight and Age for the Pilchard *Sardinops neopilchardus*

W. J. Fletcher

Western Australia Marine Research Laboratory, P.O. Box 20, N. Beach, W.A., 6020, Australia

Fletcher, W. J. 1991. A test of the relationship between otolith weight and age for the pilchard *Sardinops neopilchardus*. Can. J. Fish. Aquat. Sci. 48: 35–38.

A sample of 60 fish revealed a near-linear relationship between otolith weight and the estimated age (determined from scale and otolith annuli) for the pilchard *Sardinops neopilchardus*, with little overlap of otolith weight values between age classes. In an additional sample of 612 fish where only otolith weight and length were measured, only two clear length modes were found, but five modes using an otolith weight frequency distribution could be distinguished. The means of the five modes were similar to that predicted from the mean weights for each age class in the original sample of 60 fish. These five modes were also present in a subsample of the 612 fish, chosen such that their lengths formed a uniform distribution (i.e. no modes). These results were consistent with a direct relationship between otolith weight and age, but further validation is required before this variable can be used as the sole determinant of age.

Un échantillon de 60 poissons a montré l'existence d'une relation presque linéaire entre le poids des otolithes et l'âge estimé (déterminé selon les anneaux des écailles et des otolithes) chez la sardine *Sardinops neopilchardus*; il existe peu de chevauchement entre les classes d'âge quant aux poids des otolithes. Dans un autre échantillon comprenant 612 poissons où seuls le poids des otolithes et la longueur ont été mesurés, on ne pouvait distinguer clairement que deux modes pour ce qui est de la longueur, mais bien cinq modes distincts au moyen d'une distribution de fréquence du poids des otolithes. La moyenne des cinq modes était semblable à la moyenne prévue à partir du poids moyen pour chacune des classes d'âge dans l'échantillon de départ de 60 poissons. Ces cinq modes étaient également évidents dans un sous-échantillon des 612 poissons, choisi de façon que les longueurs étaient distribuées de manière uniforme (absence de modes). Ces résultats correspondent à l'existence d'une relation directe entre le poids des otolithes et l'âge, mais des études de confirmation s'imposent avant que cette variable puisse être employée comme unique déterminant de l'âge.

Received December 8, 1989
Accepted July 24, 1990
(JA392)

Reçu le 8 décembre 1989
Accepté le 24 juillet 1990

The accurate and efficient determination of age in fish is crucial to many of the analyses required for the management of exploited species. The methods used to assign ages to what is generally a large number of individuals are, however, usually subjective, involving the often difficult task of accurately counting the annuli on otoliths or scales. Such methods may require considerable effort to prepare each specimen, and subsequent readings are subject to both systematic and random errors in interpretation (Beamish 1979) requiring independent validation. Furthermore, considerable time is generally needed to acquire the skill necessary for consistently interpreting the material. In addition, because the criteria used to age fish are subjective, two or more readings are usually needed to verify the age assigned to an individual. Agreement among readers rarely exceeds 90% and is often much worse (e.g. Sandeman 1969).

Finding an objective measure by which the age of an individual fish could be estimated would be of considerable value. This would obviate the need for complex preparation and multiple readings and it would allow the easy exchange of information among research groups, which often differ in their interpretation using the subjective methods (Kimura et al. 1979; Boehlert 1985).

A number of ageing studies have been done using length-based cohort analyses (e.g. Samb 1988). Such methods, however, are generally only possible for fast-growing species or for the juveniles of slower growing species because the relative increase in size between age classes becomes less with age than the variation in size within each age class. Thus, overlap of lengths becomes too large and makes it virtually impossible to age individual fish.

It has been suggested that the otolith weight of a fish may be directly related to its age (Brander 1974). This hypothesis is supported by evidence that fish from slow-growing populations have been found to have relatively larger otoliths at the same length (Spratt 1972) and by experimental tests which show that the increase in size of otoliths is partially independent of the growth in length of individuals (Reznick et al. 1989). Finally, Boehlert (1985) found that a combination of otolith weight and several other objectively measured variables could be used to accurately age individuals. In this paper, the relationship between otolith weight and age is further examined.

If there is a relationship, a number of preliminary hypotheses can be tested. First, there should be little variation in otolith weights of individuals of the same age, such that the range of otolith weights for fish of different ages should not overlap greatly. Second, in a multiage population with a restricted annual spawning season, a frequency distribution of otolith weights taken from a random sample of individuals collected at one time should show a series of modes, each of which reflects a different year class. The position of these modes should be similar to that predicted from the mean otolith weight-at-age relationship.

In this paper these two hypotheses are tested for the Australian pilchard *Sardinops neopilchardus*. These should ascertain whether further tests of the relationship between age and otolith weight are worthwhile.

Materials and Methods

Test 1: Otolith Weight versus Age

Samples of pilchards were obtained from the Albany region of Western Australia (118°E, 35°S) where a major purse-seine fishery operates. The otoliths (sagittae) from a total of 60 randomly selected fish over the size range taken by the fishery, 124–186 mm length to caudal fork (LCF), were collected during a 3-mo period (June–August 1989). The otoliths were removed from the fish, washed in distilled water, dried in an oven at 60°C for 12 h, and then weighed to the nearest 0.01 mg. One of each pair of otoliths (no systematic differences between left and right otoliths were encountered) was embedded in clear epoxy resin under a glass coverslip on a black perspex slide. The rings (annuli) in each of the otoliths were analysed in the manner described by Baird (1970) and Thomas (1985) for South African pilchards to estimate age in years for each individual.

In addition, scales from the same fish were also removed, washed, and mounted between glass slides. They were examined using the criteria described for *S. neopilchardus* by Blackburn (1949, 1950) to corroborate the age ascribed using the otolith method.

The relationship between otolith weight with age and LCF was analysed among age classes using an analysis of covariance and a stepwise multiple regression (Proc GLM and Proc Reg; SAS Institute 1988). The mean weight of otoliths for each age class was also determined to provide a prediction of where the modes of otolith weights should occur, if this variable is related to age.

Test 2: Otolith Weight Frequency

Otoliths were removed from approximately 600 randomly selected individuals, all of which were captured during September 1989 in the Albany region. Each fish was measured and the otoliths removed, washed, dried, and weighed as described above. The resultant distribution of otolith weights was examined by utilising the entire sample of fish and also by examining a stratified subset of 300 individuals such that their lengths formed a uniform distribution of 30 individuals in each 5-mm size class from 130 to 170 mm LCF (i.e. no modes were present, see Fig. 2). The distributions (the modes of which were assumed to have lognormal distributions) were analysed with the assistance of "Mix" ver. 2.3 (Macdonald and Green 1988), a program designed to aid in the discrimination of frequency distributions.

Results

Test 1

In the study on the June–August samples, individuals ranged in estimated age from 2 to 6 yr old as determined by reading the annuli on the scales and otoliths (for which there was 90% agreement). For each of these ages, the range of fish lengths was large, being generally in excess of 20 mm with considerable overlap among the different age classes (>50% for all age classes greater than 2; Fig. 1). In contrast, the range of otoliths weights within an age class was relatively small with little overlap in ranges (<15%; Fig. 1).

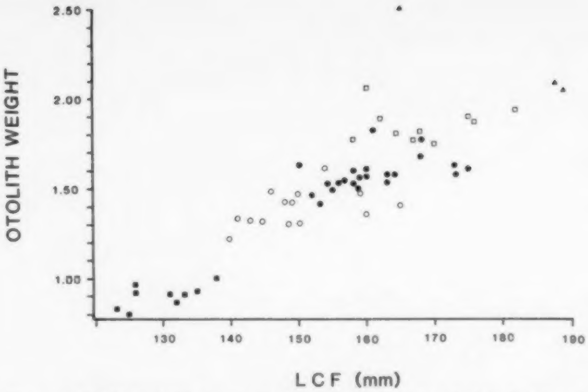

FIG. 1. Otolith weights (mg) and lengths (LCF) of individuals for age classes 2(■), 3(○), 4(●), 5(□), and 6(▲), estimated by the annuli on otoliths and scales from the June–August 1989 samples.

TABLE 1. Analysis of covariance using LCF and age, with otolith weight as the dependent variable. The interaction between LCF and age was not significant ($F = 0.058$, $P = 0.67$). One individual of age 6 which had excessive influence on the analysis was excluded.

Source	Type I MS	F	Significance	Type III MS	F	Significance
LCF	49258	666	0.0001	739	10	0.0028
Age	1631	22	0.0001	1631	22	0.0001
Error	3458					

TABLE 2. Mean (SD) weight of otoliths (mg × 100) for individuals in the five different age classes, with age estimated using three methods: (1) the rings on scales and otoliths, (2) modes in the total September sample, and (3) modes in the subsample of individuals which formed a uniform length distribution. Age class 6 for both modal groups did not include the outliers past 2.4 mg.

Age class	Otolith/scale estimate (June–August)	Model groups, total sample (September)	Modal groups, subsample (September)
2	81.3 (6.6)	90.0 (13)	88.2 (13)
3	130.2 (10.3)	135.3 (10)	131.0 (9)
4	148.1 (9.7)	152.4 (10)	151.1 (9)
5	176.0 (9.8)	175.8 (10)	174.9 (9)
6	210.2 (25)	205.9 (12)	205 (10)

An analysis of covariance indicated that age still made a significant contribution to explain the variation in otolith weight even when length had been accounted for (Table 1), thereby supporting the hypothesis that otolith weight and age are closely related. Similarly, using a stepwise multiple regression, the amount of variance explained, as measured by the partial regression coefficients, by age and length was 89 and 1%, respectively. These results confirmed that age was a stronger predictor of otolith weight than was length.

The mean weights of otoliths for each age class increased in an almost linear fashion with age; importantly, a substantial increase continued even in the older age classes (Table 2). The

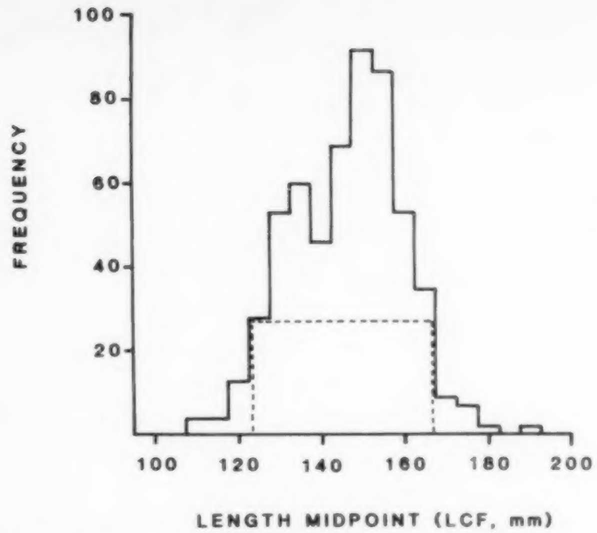

FIG. 2. Length frequency distribution of the total sample (solid line, $n = 612$) and subsample (broken line, $n = 289$) of pilchards used in the September 1989 otolith weight analysis.

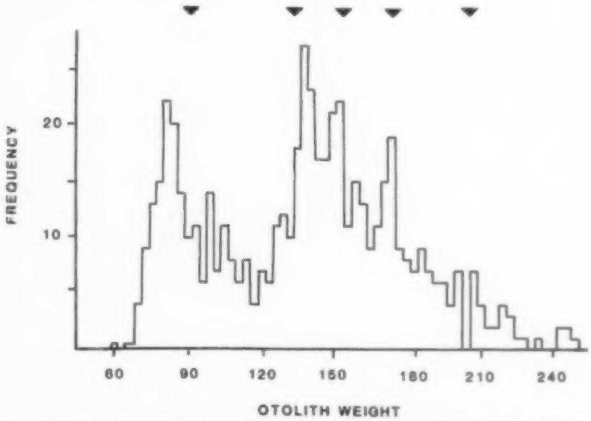

FIG. 3. Otolith weight (mg × 100) fequency distribution of pilchards from the total sample of pilchards caught in September 1989 ($n = 612$). Arrowheads indicate the position of the mean value for each mode.

closeness of age classes 3 and 4 probably reflects a variation in the time of spawning between some years. Similarly, the gap between ages 5 and 6 may be an artifact of the low sample numbers.

Test 2

The sample from September 1989 consisted of fish with two partly separated length modes (Fig. 2). The otolith weight frequency distribution was significantly different from the length distribution (KS test, $D = 0.19$, $P < 0.01$). The best fit for the frequency distribution (Fig. 3) for otolith weights was when five modes were assumed (chi-square = 65, $P > 0.05$); all other combinations resulted in the fitted distribution being significantly different from the observed distribution. The mean values of these modes, as determined by the "Mix" program, were similar to those predicted by the mean values of the five age classes of the initial analysis (Table 2). In general, the slightly larger values in the later September sample could be expected because the otoliths should have grown between the two periods.

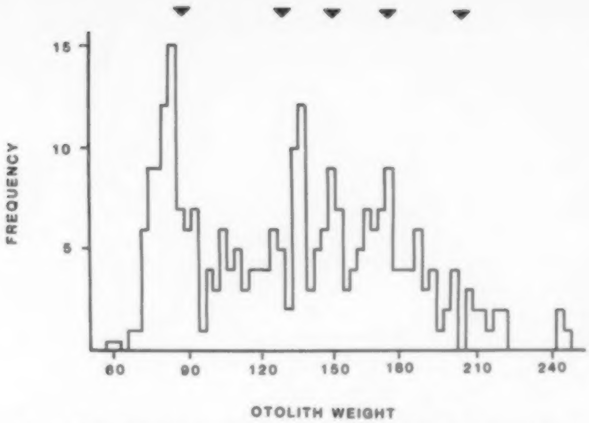

FIG. 4. Otolith weight (mg × 100) frequency distribution for the uniform subsample of pilchards (30 individuals in each 5-mm length class from 130 to 170 mm LCF) from the September sample ($n = 289$). Arrowheads indicate the position of the mean value for each mode.

A further test of the independence of otolith weight from fish length was made by analysing the otolith weight distribution from the subsample of fish with the uniform distribution of lengths. Despite there being no modes in lengths, the multimodal situation persisted for otolith weight (Fig. 4); again, five modes gave the best fit (chi-squre = 56.7, $P > 0.05$). Furthermore, the mean values of these modes were still consistent with the predicted values (Table 2).

Discussion

While there is some relationship between otolith weight and LCF (Fig. 1; Table 1), the otolith weight of an individual Australian pilchard is not totally dependent upon overall length. If otolith weight was merely dependent upon length, it would be expected that the distributions of these two variables would be similar. Instead, the distributions of otolith weights were significantly different from the length structures of the samples.

The independence of otolith weight from fish length has been found elsewhere. For the Californian anchovy *Engraulis mordax*, it was found that some slow-growing populations had otoliths which were relatively bigger than similarly sized fish from fast-growing populations (Spratt 1972). This information was, however, only used to help separate the populations. Similarly, Reznick et al. (1988) experimentally influenced the growth rate of guppies, *Poecilia reticulata*, and found that slower growing individuals had relatively larger otoliths than faster growing fish, despite coming from the same population. They cited eight other studies where less rigorous tests showed similar findings. Nonetheless, most of these studies did not elaborate upon a major implication of these results that otolith weight could possibly be used as a determinant of age.

The results of the present study support the hypothesis that there is a relationship between otolith weight and the age of individuals for the pilchard *S. neopilchardus*. The evidence includes the small overlap of otolith weights among age classes as determined by the annuli. The mean values increased significantly even for the older age classes, which is not generally evident in other structures (e.g. lengths). Moreover, distinct modes in otolith weight could be discriminated even when there was no modal structure in the length distribution of the fish. The means of these modes were in close agreement with those predicted independently by the ageing study.

The use of otolith weights has already been suggested as an objective method for ageing the rockfish *Sebastes diploproa* by Boehlert (1985). In his study, a combination of factors was used in a multiple regression technique to age fish up to 30 yr old, which agreed closely with the ages determined from sectioned otoliths. For *S. neopilchardus*, it seems that otolith weight alone may be sufficient to discriminate ages with a similar level of accuracy to scale and otolith reading. At the very least, it could be used as a check on the ring counts from otoliths and scales.

The findings presented here are only preliminary; before otolith weights can be used as the sole ageing technique, further validation is required. This will involve the validation of ageing by annuli and establishing that the modes of otolith weights persist and progress through time. Nonetheless, the results to date are promising and it may be possible that this objective measure may take ageing out of being what Williams and Bedford (1974) described as more art than science.

Acknowledgements

Thanks are expressed to the fishermen and procesors of Albany for providing the fish. Sampling was done with the expert assistance of R. Tregonning, S. Blight, and M. Rossbach. Figures were photographed by S. Blight. The manuscript was improved by reviews from M. Moran, R. Lenanton, N. Caputi, N. Morrisey, J. Penn, N. Hall, and two referees.

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Dynamics of Cadmium, Lead, and Zinc Exchange between Nymphs of the Burrowing Mayfly *Hexagenia rigida* (Ephemeroptera) and the Environment

Landis Hare

Université du Québec, INRS-Eau, C.P. 7500, Sainte-Foy (Québec) G1V 4C7, Canada

Erwan Saouter

Laboratoire d'Écologie fondamentale et Écotoxicologie, Université de Bordeaux I, 33405 Talence Cedex, France

Peter G. C. Campbell and André Tessier

Université du Québec, INRS-Eau, C.P. 7500, Sainte-Foy (Québec) G1V 4C7, Canada

and Francis Ribeyre and Alain Boudou

Laboratoire d'Écologie fondamentale et Écotoxicologie, Université de Bordeaux I, 33405 Talence Cedex, France

Hare, L., E. Saouter, P. G. C. Campbell, A. Tessier, F. Ribeyre, and A. Boudou. 1991. Dynamics of cadmium, lead, and zinc exchange between nymphs of the burrowing mayfly *Hexagenia rigida* (Ephemeroptera) and the environment. Can. J. Fish. Aquat. Sci. 48: 39–47.

Radioisotopes of cadmium, lead, and zinc added in trace amounts to lake sediments were used to measure the uptake and efflux of these metals from various body parts of nymphs of the burrowing mayfly *Hexagenia rigida* (Ephemeroptera). Total metal concentrations in *Hexagenia* and its environment were held constant. A simple model permitted the estimation of rate constants that were used to generate model curves which corresponded closely to the measured trends in trace metal uptake and efflux. There was no measurable accumulation of radioisotopes in gill tissues, suggesting that the gills were not the major organ of metal uptake in *Hexagenia* in this experiment. On the other hand, net uptake of ^{109}Cd and ^{65}Zn by the gut exceeded that by all other body parts in both quantity and concentration terms, suggesting that the primary source of these metals to *Hexagenia* is sediment consumed as food. The rate of exchange of ^{65}Zn was slower than that of ^{109}Cd. ^{210}Pb differed from the other two metals in that it was not detected in the gut, but was found mainly on the body surface.

Des radioisotopes du cadmium, du plomb et du zinc ont été ajoutés en très petites quantités à des sédiments lacustres pour mesurer l'influx et l'efflux de ces métaux traces dans différentes parties du corps de la larve de l'éphémère fouisseuse *Hexagenia rigida* (Ephemeroptera) dans des conditions de quasi état stationnaire (i.e. dans des conditions où les concentrations environnementales et animales de métaux traces demeuraient inchangées). À l'aide d'un modèle simple on a estimé les constantes de vitesse pour la prise en charge et l'élimination des métaux. La dynamique d'échange, telle que simulée avec ces constantes de vitesse, se comparait avantageusement avec l'influx et l'efflux mesurés. Il n'y a pas eu d'accumulation mesurable des radioisotopes dans les branchies, ce qui suggère que les branchies ne sont pas l'organe majeur d'entrée de ces métaux chez *Hexagenia* dans cette expérience. La concentration et la quantité de ^{109}Cd et de ^{65}Zn dans l'intestin excédaient celles des autres parties du corps, ce qui suggère que la source principale de ces métaux pour *Hexagenia* est la nourriture (sédiments). Le taux d'échange du ^{65}Zn était plus faible que celui du ^{109}Cd. Le ^{210}Pb n'a pas été détecté dans l'intestin mais a été retrouvé largement adsorbé sur le corps des larves.

Received February 15, 1990
Accepted July 23, 1990
(JA473)

Reçu le 15 février 1990
Accepté le 23 juillet 1990

Organisms are used increasingly as contaminant biomonitors in aquatic systems. The choice of an appropriate biomonitor requires a knowledge of the rates of exchange of contaminants between the candidate organism and its environment. In slow-growing organisms, the biological half-time of pollutants may be on the order of many months (e.g. large bivalve molluscs; Luten et al. 1986; Marigomez and Ireland 1989). Such organisms would be of limited use in short-term biomonitoring studies. In contrast, small, active, fast-growing species, such as insects, have the potential to respond much more rapidly to changes in ambient pollutant concentrations. In the laboratory, aquatic insects achieve within a few days apparent steady state with various concentrations of dissolved mercury (Saouter et al. 1989) and cadmium (Clubb et al. 1975; Dressing et al. 1982; Yamamura et al. 1983). Exchange rates in nature are, however, little known.

Rates of exchange of trace metals between benthic animals and their surroundings are often measured at concentrations far higher than those found in nature. Exchange mechanisms could differ at high and low metal concentrations. In addition, deposit feeders are often studied in water alone which precludes feeding (and uptake of metals by this route) and could alter the uptake pattern of trace metals from water (Luoma 1983). In general, sediment-bound trace metals are less easily taken up by organisms than those in water; however, high sediment metal concentrations can result in a greater uptake from this source (Luoma 1983). Especially lacking are measurements of pollutant exchange between *freshwater* invertebrates and their environment.

Measurements of contaminant dynamics are also required for the assessment of losses from animals during the laboratory depuration of gut contents prior to contaminant analysis (Hare et al. 1989). The placement of active burrowing organisms in conditions of stress (without sediments) and in the near absence of a pollutant could lead to pollutant loss and a corresponding underestimate of organism pollutant concentrations.

The objective of the present study was to measure the exchange rates (influx and efflux) of trace metal contaminants (cadmium, lead, and zinc) between a freshwater benthic insect, the sediment-feeding mayfly *Hexagenia rigida* (McDunnough) (Ephemeroptera), and its environment. Radioisotopes of the trace metals, added in small quantities to sediments in laboratory microcosms, were used to follow trace metal movements. Exchange rates were measured in individual body parts in order to identify the sites of trace metal storage and exchange and to determine the probable environmental source of pollutants, i.e. from water or food.

Methods

Preparation of Radioactive Sediment and Microcosms

Sediment was collected in January 1987 from a site inhabited by *Hexagenia* (depth 15 m) in a lake subject to aerial fallout from a smelter located 30 km to the west at Rouyn-Noranda in western Quebec (Lake Joannès, 48°11′N, 78°41′W). In the laboratory, approximately 600 μCi (1 μCi = 37 kBq) of each of the radioisotopes ^{109}Cd, ^{210}Pb, and ^{65}Zn was added to 15 L of sediment, which was then mixed using a paddle fitted onto a power drill. The efficacy of the mixing technique was confirmed by the small variation measured among counts of radioactivity in sediment samples (coefficients of variation $\leq$4% among 10 sediment cores). The sediments were kept in a closed container for 10 mo prior to use in order to obtain uniform specific activities among the various sediment fractions. Since this long equilibration time exceeded the half-life of ^{65}Zn, an additional 600 μCi of ^{65}Zn was added 2 wk prior to the start of the biological uptake experiment to ensure that measurable levels would be obtained in nymphs.

One half litre of radioactive sediment was placed in each of 27 cylindrical (20 cm high, 12 cm diameter), transparent plastic containers ("Frig-O-Seal") which were subsequently filled with 1.5 L of spring water (20 and 5 ppm Ca and Mg, respectively). Nine additional containers were prepared in the same manner using unlabeled sediment from the original batch, for later use in the measurement of radioisotope efflux. All containers were kept covered in the dark at 20°C throughout the experiment. Oxygen levels in the water were maintained at near 100% saturation by constant aeration and pH varied little (7.0–7.5). The containers were left for 2 wk prior to the introduction of nymphs to allow for the oxidation of surficial sediments, as occurs in nature.

The use of radioisotopes allowed the study of trace metal movements among the body parts of the insects without measurably increasing environmental metal concentrations. In addition, variability was reduced as all individuals began the experiment with only background radiation levels.

Rearing and Introduction of Nymphs

Eggs of *H. rigida* taken from adults collected near Lake Winnipeg, Manitoba (supplied by M. Freisen, Freshwater Institute, Winnipeg, Man.), were hatched and the nymphs reared (according to Freisen 1981) at Bordeaux, France, in sediments collected from the nearby Garonne River. After transport to Canada, nymphs were maintained in sediments from Lake St. Joseph, Que. (46°55′N, 71°40′W) for 1 mo prior to use. Nymphs were sieved from the sediment and grouped into four weight classes (ranging from 15 to 60 mg live weight), and one individual from each class was placed into each of the 27 microcosms containing radioactive sediments. This procedure insured that there was little difference among replicates both in terms of mean nymph size (mean wet weight ($\pm$SD) = 35.7 $\pm$ 1.7 mg) and the range and distribution of nymph sizes (mean coefficient of variation of nymph wet weights ($\pm$SD) = 35.1 $\pm$ 2.6%).

Influx and Efflux Experiments

The concentration of trace metals in nymphs was measured in three replicate containers at each of 2, 4, 8, 16, 32, and 50 d (influx experiment). After 50 d of exposure, nymphs in the nine remaining radioactive microcosms were removed and redistributed in the nine microcosms containing unlabeled sediment. A uniform size distribution of nymphs was maintained as described above. Trace metal concentrations in the nymphs were then measured after 3, 8, and 16 d (efflux experiment).

On each sampling day, three containers were selected at random and radioactivity was measured, as described below, in both sediment (three cores) and water (10-mL samples, unfiltered and filtered with a 0.4-μm Nuclepore filter). In order to measure the distribution of metals among sediment fractions, the surficial layers (1 cm) of cores collected from containers sampled at days 4, 16, and 50 were subjected to a sequential extraction procedure based on the methods of Tessier et al. (1979) and Belzile et al. (1989). This procedure involves the use of a series of reagents of increasing strength that serve to extract trace metals from increasingly refractory sediment fractions.

Nymphs were removed from the sediments by sieving. Of the four nymphs introduced initially, three or four remained alive at the time of sampling with the exception of one microcosm on day 32 which contained only one nymph (not included in data analyses). Radioactive gut contents were eliminated by placing nymphs for 3 h in beakers containing nonradioactive Lake Joannès sediment to which fluorescent dye particles had been added. Nymphs were removed and examined under a microscope and ultraviolet light to ensure that dye particles were visible along the entire length of the gut.

For dissection, individuals were placed in a small amount of deionized water, an incision was made between the thorax and the head to sever the gut and immobilize the animal, and the body surface was gently scraped as clean as possible of adhering deposits. The gills were removed and the abdomen was opened ventrally in order to remove the gut. For the few individuals that had not depurated the radioactive sediments, the gut was opened to permit their removal. Each body part (gills, gut, remainder) was placed on a small preweighed piece of acid-washed Teflon, frozen at −40°C, dried in a lyophilizer, and weighed. Details of the dissection procedure are given in Hare et al. (1989). The weight of the gut, without contents, was estimated from body weight using a relationship developed for *Hexagenia limbata* (Hare et al. 1989), i.e. $Y = 0.0952X - 0.0482$, where Y and X are the dry weights (milligrams) of the gut and body, respectively ($r^2 = 0.97$, $n = 10$; for body weights from 0.5 to 15.0 mg). Losses of radioactive metal during dissection represented <5% of the total trace metal burden of all individuals measured on day 50.

Samples were digested individually in screw-cap Teflon vials in concentrated Aristar-grade nitric acid (100 µL·1.0 mg body weight^{-1}) in a water bath at 70°C for 48 h. Digested samples were transferred to either an acid-washed polypropylene 1.5-mL snap-cap scintillation vial (small samples) or a 10-mL polystyrene test tube (larger samples) and diluted with deionized water to a final acid concentration of 16.7% (5:1 v/v). Deionized water for all uses was obtained from a Milli-Q3RO/Milli-Q2 system (Millipore Corp.).

Analyses

Radioactive trace metals were measured in an "LKB Wallac 1282 Compugamma" gamma particle counter (NaI(Tl) well type). Correction was made for radioactive decay and for background radiation. Only net values >3 SD of the mean of 10 blank samples were retained. Counts of ^{109}Cd and ^{210}Pb were corrected for interference from each of the other radioisotopes present (counts of ^{65}Zn were unaffected by the other metals). Samples of gill tissue did not in most cases exceed background values, even when the gills of all individuals from one sampling day were pooled. Counts of ^{210}Pb in the gut were also below background once corrected for interference from the other trace metals. Counting efficiencies (^{109}Cd 60%, ^{210}Pb 3%, ^{65}Zn 9%) were determined in order to convert counts per minute (CPM) values to disintegrations per minute (DPM).

To determine if steady-state conditions prevailed during the experiment, total trace metal concentrations in nymphs were measured by atomic absorption spectrophotometry, either by flame atomization (Zn; Varian AA-575) or in a graphite tube furnace (Cd; Varian AA-1275 with GTA-95 graphite tube atomizer). Correction was made for the contribution of gut contents to trace metal concentrations in gut tissues using the calculation method given in Hare et al. (1989). Measures of similar-sized samples of a certified reference material (lobster hepatopancreas, TORT-1, National Research Council of Canada, Ottawa, Ont.) varied little over time (coefficient of variation 3–7%; $n = 10$ for each metal) and were close to the expected certified values (within 3.5–6.0% of expected means; $n = 10$ for each metal). Total lead could not be accurately measured with the digestion method used.

Results

Distribution of Radioisotopes in Sediments and Water

A radioisotope can be used to follow the movement of its nonradioactive counterpart provided that the system is at steady state, both over time and with respect to the distribution of the isotopes. This is a necessary condition if the biological uptake of the two forms is to be comparable.

There was little or no change in specific activity *within* each sediment fraction during the course of the experiment, suggesting that the bioavailability of the isotopes of each metal varied little over time (Fig. 1). In addition, isotope distributions among sediment fractions were largely at steady state by the beginning of the experiment, as evidenced by the similarity among the specific activities of the more weakly bound sediment fractions (Fig. 1). The strength of metal/sediment associations approximately increases with increasing fraction number. In contrast, in the most tightly bound fraction (5) the specific activities of Pb and Zn were low and ^{109}Cd was not detectable. This indicates that little radioactive metal had been incorporated into fraction 5 which is mainly not bioavailable. In fraction 4 (extraction with $HNO_3/HClO_4$), the specific activity of Zn was somewhat lower than in preceding fractions, a consequence perhaps of the late addition of a portion of the

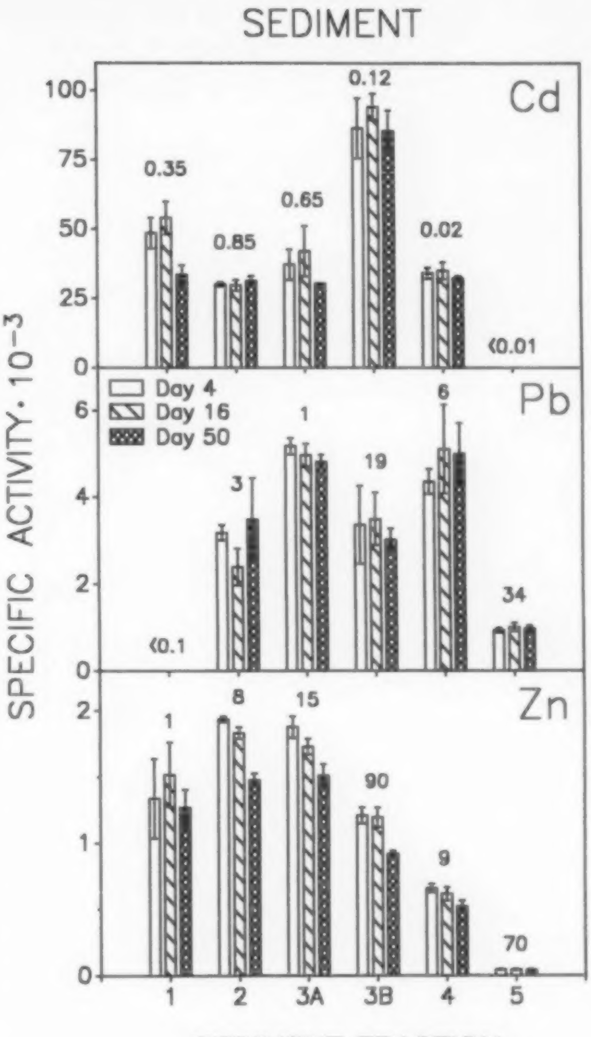

FIG. 1. Mean (±SD) specific activities (DPM radioactive metal·µg total metal^{-1}) of Cd, Pb, and Zn in fractions of surficial sediments collected on days 4, 16, and 50 of the experiment. Mean total metal concentrations (µg·g^{-1}) are indicated above the histograms for each fraction. Fractions correspond to successive extractions of the sediment with (1) $MgCl_2$, (2) acetate buffer, (3A) $NH_2OH{\cdot}HCl$ at room temperature, (3B) $NH_2OH{\cdot}HCl$ at 96°C, (4) H_2O_2/HNO_3, and (5) $HNO_3/HClO_4$.

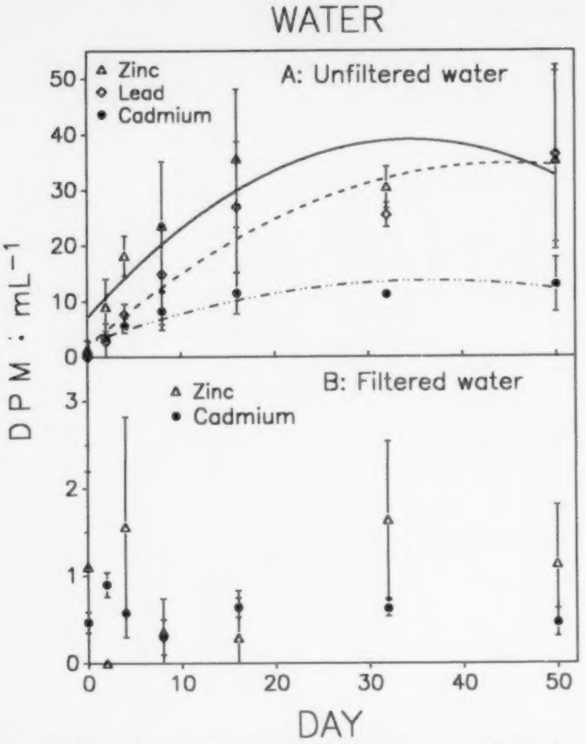

FIG. 2. Changes over time in the concentrations ($\bar{x}$ ± SD) of radioactive trace metals in (A) unfiltered and (B) filtered microcosm water. Pb was not detected in filtered water.

radioactive Zn. In fraction 3b (extraction with $NH_2OH \cdot HCl$) the specific activity of Cd was anomalously high. However, since the bulk of the Cd was associated with previous fractions (85% of ^{109}Cd in fractions 1–3a), this difference probably had little biological importance. These observations suggest that the radioisotopes had attained a steady state with respect to their nonradioactive counterparts.

All of the radioisotopes were distributed uniformly with depth in sediment cores collected both early (2 d) and late (32 d) in the exposure period (coefficient of variation <7% among all core subsamples from three depths on both sampling days). This suggests that nymphs feeding at different depths in the sediment would not have been exposed to different total radioisotope concentrations.

The quantity of radioactive sediment suspended in the water increased during the early part of the experiment (Fig. 2A), as a consequence of the burrowing and feeding activities of the nymphs. However, the radioactivity of filtered water did not increase (Fig. 2B), indicating that trace metals adsorbed onto particles in the water did not readily pass into solution.

Uptake and Depuration of Trace Metals

As expected, total concentrations of Cd and Zn in body parts did not show significant trends over time (Fig. 3). Thus the radioactive trace metal dynamics observed can be considered to be representative of those at steady state.

Animals took up the radioisotopes of Cd, Pb, and Zn rapidly during the early part of the experiment, after which time net uptake slowed as an apparent steady state between radioisotope influx and efflux was reached (Fig. 4). The general form of the net uptake curves is similar for the gut and for the body (animal minus gut and gills) for both ^{109}Cd and ^{65}Zn. Radioactive Pb in the gut was below the detection limit, as were all three radioisotopes in the gills.

The concentrations of Cd and Zn were always much higher in the gut than in the body (Fig. 3 and 4), suggesting that the major entry site of Cd and Zn is the gut. Furthermore, the ratio of ^{65}Zn in the gut to that in the body declined during the first 1–2 wk of the experiment (Fig. 5), suggesting an initial entry via the gut and a subsequent transfer to the body. Such a trend was not observed for Cd (Fig. 5), suggesting that there was little movement of Cd from the gut to the body or, and perhaps more likely, that such movement occurred but was too rapid to be detected given the time scale of the experiment. Gut metal concentrations greatly exceeded those in the sediment, i.e. the ratios of gut/sediment concentrations at near zero net uptake were Cd 45 and Zn 15. Pb was not detected in the gut. In contrast, body metal concentrations were somewhat lower than those in the sediment (ratio body/sediment: Cd 0.35, Zn 0.50, and Pb 0.20).

Animals labeled for 50 d and then transferred to nonradiolabeled sediments lost radioactive trace metals from both the gut and the body (Fig. 6).

Discussion

Kinetics of Metal Exchange

Nymphs readily gained and lost radioisotopes in the two phases of the experiment, indicating a continuous exchange of Cd, Pb, and Zn between *Hexagenia* and its environment. A model based on first-order kinetics for both influx and efflux can be used to describe these exchanges for either the body or the gut. According to this simple model, net uptake can be written as follows:

$$(1) \quad \frac{d[\mathrm{M}]_a}{dt} = k_I \cdot [\mathrm{M}]_e \text{ (influx)} - k_E \cdot [\mathrm{M}]_a \text{ (efflux)}$$

where k_I and k_E are first-order rate constants for the influx and efflux of trace metals, respectively, and $[\mathrm{M}]_e$ and $[\mathrm{M}]_a$ represent the concentrations of trace metal in the external environment and in animal tissues, respectively. Equation (1) holds for the "cold" metal (M) or for the radioisotopes (*M). The values of the constants k_E and k_I can be estimated using the experimental data. First, k_E is obtained from the efflux experiments for which Eq. (1) reduces to

$$(2) \quad \frac{d[{}^*\mathrm{M}]_a}{dt} = -k_E \cdot [{}^*\mathrm{M}]_a$$

since $[{}^*\mathrm{M}]_e$ is virtually 0 in the efflux experiment. The latter equation is readily integrated to

$$(3) \quad [{}^*\mathrm{M}]_a = [{}^*\mathrm{M}]_a^0 \, e^{-kEt}$$

where the index zero refers to initial conditions. The values of k_E obtained from regression of ln $[{}^*\mathrm{M}]_a$ versus time (t) are given in Table 1.

The rate constants k_I can be estimated from the data obtained early in the influx experiments, i.e. from those data for which $[{}^*\mathrm{M}]_a$ is low enough so that the second term in Eq. (1) is much smaller than the first one. Equation (1) then reduces to

$$(4) \quad \frac{d[{}^*\mathrm{M}]_a}{dt} = k_I \cdot [{}^*\mathrm{M}]_e = k'_I$$

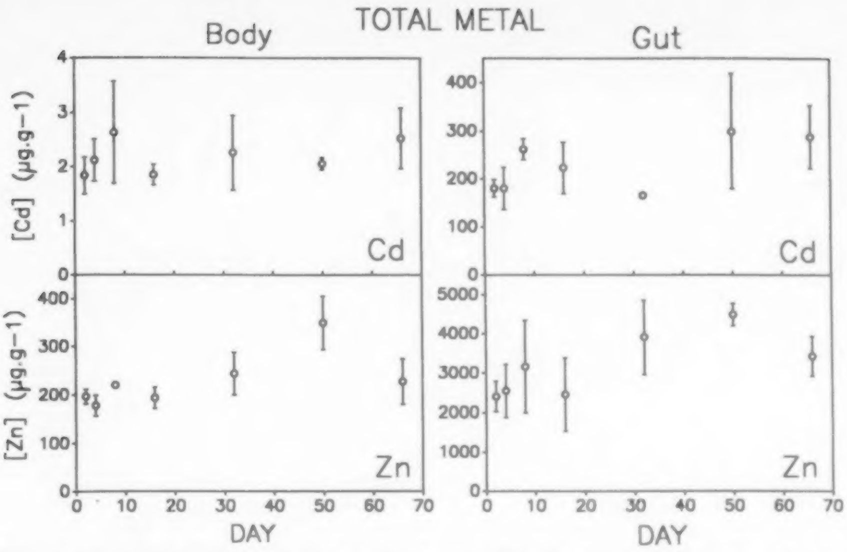

FIG. 3. Changes throughout the course of the experiment in the total concentrations ($\bar{x}$ ± SD; μg·g dry weight^{-1}) of Cd and Zn in the body and gut of *Hexagenia*. Nymphs were transferred from radiolabeled to nonradiolabeled sediments on day 50. The mean and standard deviations of values for three microcosms (two on day 32) are presented on each sampling day.

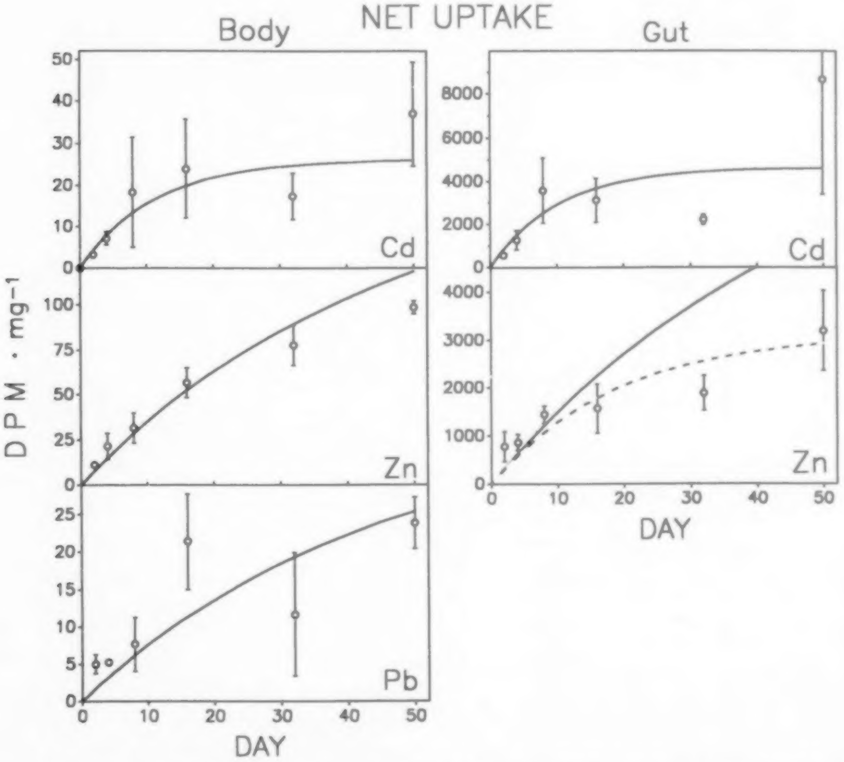

FIG. 4. Net uptake over time of radioactive Cd, Zn, and Pb in the body and gut of *Hexagenia* held in radiolabeled lake sediments for 50 d. The mean and standard deviations of concentrations (DPM·mg dry weight^{-1}) for three microcosms (two on day 32) are presented on each sampling day. Model curves were generated according to Eq. (6); curves for Zn in the gut were generated either with k_E derived from experimental data from days 0–16 (solid curve) or from days 0–8 (broken curve).

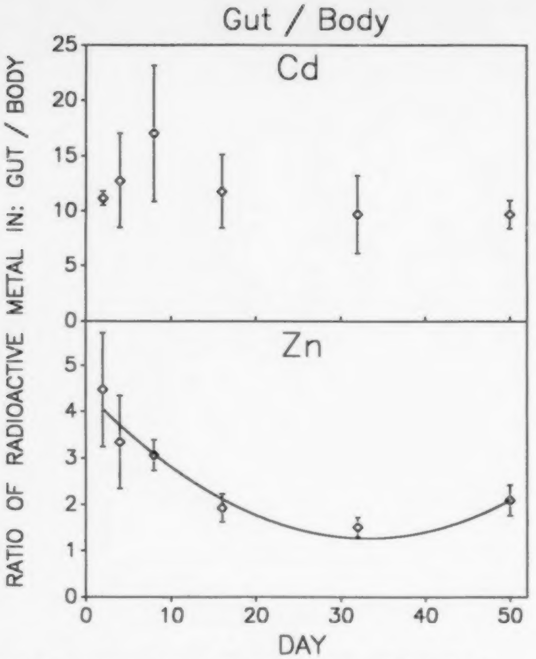

FIG. 5. Changes over time in the ratios of the quantities of radioactive Cd and Zn in the body to those in the gut of *Hexagenia* held in radiolabeled lake sediments for 50 d. The mean and standard deviations of values for three microcosms (two on day 32) are presented on each sampling day. A curvilinear regression for Zn data was generated by the software package Sigmaplot.

Since $[^*M]_e$ was almost constant during the experiment because the animals took up only a tiny fraction of the radioisotopes added to the system, the influx has been represented as a single term k'_I in Eq. (4). Integration of Eq. (4) leads to

$$(5)\quad [^*M]_a = k'_I\, t.$$

As expected from Eq. (5), approximately straight lines are observed early in the uptake experiments (days 0–8, Fig. 4), the slopes of which were taken as estimates of the apparent rate constant k'_I (Table 1). Model curves were generated (Fig. 4 and 6) from the k'_I and k_E values given in Table 1 and the appropriate values of $[^*M]_a^0$ by solving Eq. (1) for $[^*M]_a$:

$$(6)\quad [^*M]_a = k'_I / k_E - (k'_I / k_E - [^*M]_a^0)e^{-kEt}$$

The net uptake of Zn by the gut was best described by a model curve generated with a value of k_E estimated without the last data point in the efflux experiment (i.e. day 16, broken curves in Fig. 4 and 6).

The internal consistency of the values of k'_I and k_E can be checked in the following manner. By definition, $[^*M]_a$ at steady state is constant over time and thus Eq. 1 becomes

$$\frac{d[^*M]_a}{dt} = k_I\cdot[^*M]_e\text{ (influx)} - k_E\cdot[^*M]_a\text{ (efflux)} = 0$$

or

$$(7)\quad k_I\cdot[^*M]_e = k_E\cdot[^*M]_a^{ss}$$

where $[^*M]_a^{ss}$ is the steady-state metal concentration in a body part. Since $k_I\cdot[^*M]_e$ was defined in Eq. (4) as k'_I, Eq. (7) can be rewritten as

$$(8)\quad \frac{k'_I}{k_E} = [^*M]_a^{ss}.$$

The similarity of the ratios of the influx and efflux constants, as given in Table 1, to the measured steady-state concentrations in the various body parts, as shown in Fig. 4, is reassuring. As a final check, the ratios of the influx and efflux constants (i.e. $[^*M]_a^{ss}$) can be divided by the sediment radioisotope concentrations ($[^*M]_{sed}$) to obtain steady-state bioconcentration factors (Table 1). These values should be comparable with bioconcentration factors of "cold" metals (ie. $[M]_a/[M]_{sed}$), as given in Table 1. Although the two bioconcentration factors are of similar magnitude, the radioisotope bioconcentration factors are consistently lower than those for the "cold" metal, suggesting that a portion of the total metal present initially in the nymphs had a slower exchange rate with the environment than did the remainder. The notion of internal "pools" of a trace metal differing in exchange rates has been proposed for several marine invertebrates (e.g. Bryan and Hummerstone 1973). The location (intra- versus extracellular, e.g. Andersen and Baatrup 1988) and form (e.g. granules, Brown 1982; protein bound, Yamamura et al. 1983) in which slowly exchanging pools of metals might be sequestered in aquatic insects are not yet known.

The biological half-times ($t_{1/2}$) of each metal in each body part can be estimated from Eq. (3). These correspond to about 1 wk for Cd and longer times (2 wk to 1 mo) for Zn and Pb (Table 1). These data suggest that if live organisms are left in metal-free water for several days in order to remove contaminated gut contents (Hare et al. 1989), losses of Cd might be substantial while those of Zn and Pb should be less.

In general, the model curves fit the experimental data well, suggesting that this simple model is sufficient to characterize the rates of net metal uptake by *H. rigida* in the experimental system. Apparent steady states should be reached in 1–3 mo. This suggests a time frame for the turnover of the pools of exchangeable trace metals under the experimental conditions which were comparable with those in the littoral zone of a temperate lake during summer. It is to be expected that at other temperatures, metabolic and feeding rates would change (Zimmerman and Wissing 1978) with concomitant effects on trace metal exchange rates (Mishima and Odum 1963; Van Dolah et al. 1987; Douben 1989).

It is acknowledged that the direct extrapolation of laboratory results to nature must be made with caution, e.g. mean nymph weight did not increase during the present experiment, suggesting that the animals may have been stressed. In nature, trace metal exchange rates could also vary with changes in environmental conditions (Van Dolah et al. 1987), the concentrations of other metals (Wicklund et al. 1988), as well as with an individual organism's history of pollutant exposure (Bryan and Hummerstone 1971). Furthermore, extrapolations from one metal to another must be made with caution, since the dynamics of metals such as Zn, which is an essential micronutrient, are likely to differ from those of other metals such as Cd and Pb, which are metabolic poisons (Amiard et al. 1987; Rainbow 1988).

Metal Dynamics in the Gills and Gut

Exchange rates were measured for individual body parts in order to estimate trace metal sources; metals in the gills or gut

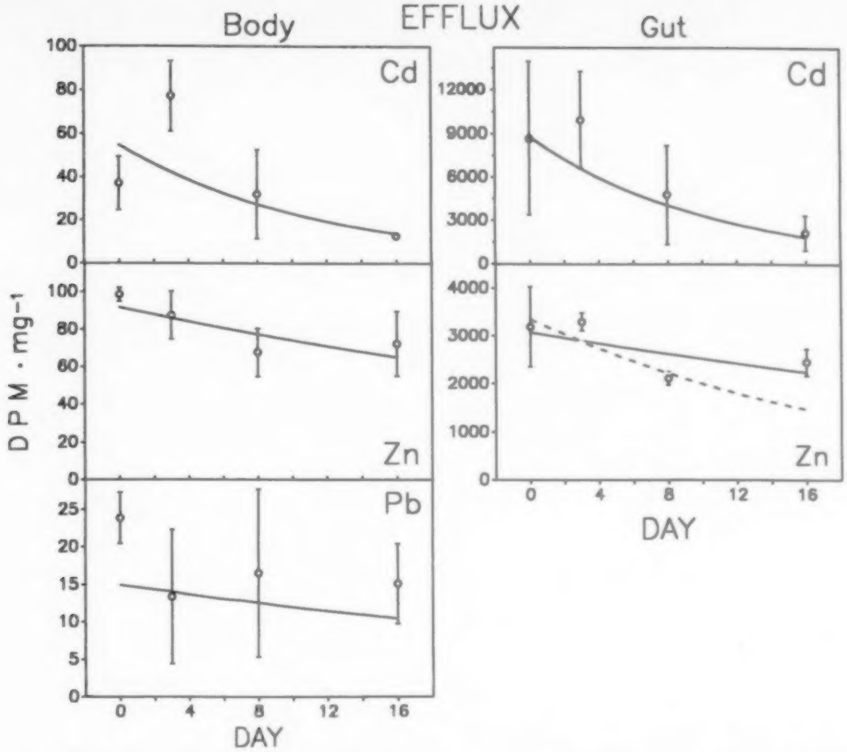

FIG. 6. Efflux of radioactive Cd, Zn, and Pb from the body and gut of *Hexagenia* in nonradiolabeled lake sediment. Nymphs had been previously held in radiolabeled lake sediments for 50 d. The mean and standard deviations of concentrations (DPM·mg dry weight^{-1}) for three microcosms are presented on each sampling day. Model curves were generated according to Eq. (3); curves for Zn in the gut were generated either with k_E derived from experimental data from days 0–16 (solid curve) or from days 0–8 (broken curve).

TABLE 1. Estimated values (±SE) of various model terms for trace metal exchange in nymphs of *H. rigida*. Sediment trace metal concentrations, $[\mathrm{M}]_{sed}$ and $[^*\mathrm{M}]_{sed}$, are the sum of metals in sediment fractions 1–4 (i.e. "residual" trace metals (fraction 5) omitted).

		Body			Gut		
Term	Symbol	Cd	Zn	Pb	Cd	Zn	Zn^a
Efflux rate constant (d^{-1})	k_E	0.088 (±0.028)	0.022 (±0.011)	0.022 (±0.051)	0.098 (±0.034)	0.020 (±0.010)	0.052 (±0.021)
Biological half-time (d)	$t_{1/2}$	8	32	31	7	35	14
Apparent influx rate constant (DPM·mg^{-1}·d^{-1})	k'_I	2.3 (±0.7)	3.9 (±0.6)	0.85 (±0.24)	450 (±90)	160 (±30)	160 (±30)
k'_I/k_E		25	175	38	4600	8000	3100
$(k'_I/k_E)/[^*\mathrm{M}]_{sed}$		0.35	1.15		60	50	20
$[\mathrm{M}]_a/[\mathrm{M}]_{sed}$		1.1	1.9		110	27	27

^{a}Alternate values for gut Zn are based on data from days 0, 3, and 8 only (i.e. day 16 omitted).

are likely to have been taken up from water or food, respectively. Uptake from these sources is thought to be both independent and additive (Giesy et al. 1980; Hattum et al. 1989). The large volumes of water passing over the gills of *Hexagenia* could favour the uptake of trace metals from water. However, radioactivity in the gills of *Hexagenia* never exceeded back-

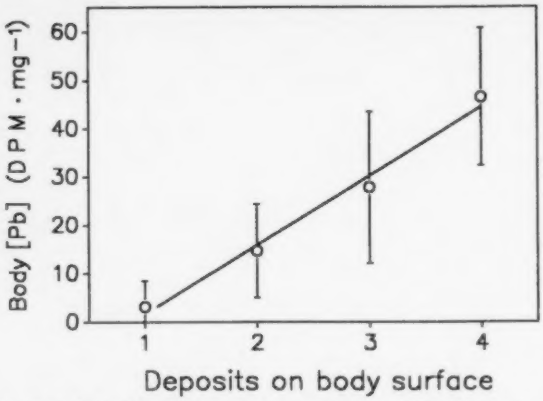

FIG. 7. Relationship between the concentration of Pb (DPM·mg dry weight^{-1}) associated with the body of *Hexagenia* and the age of its exoskeleton, as estimated from the extent of deposits on the body surface using a visual scale from 1, no deposit, to 4, a heavy deposit ($r^2 = 0.64$, $n = 59$ organisms sampled on days 16–66, $\bar{x} \pm$ SD).

ground levels of Cd, Pb, and Zn. In nature, aquatic insects also tend to have low concentrations of these trace metals in respiratory structures (L. Hare, A. Tessier, and P. G. C. Campbell, in review).

The radioactivities of Cd and Zn in the gut of *Hexagenia* were greater at all times than those in the body, both in quantity and in concentration. Furthermore, the influx rate constants are about 2 orders of magnitude higher for the gut than for the body (Table 1), suggesting that gut tissues are the major site of storage and entry of these metals and that they are taken up largely from sediment consumed as food.

External versus Internal Localisation of Metals

Negative relationships between insect body size and trace metal concentrations have been taken to indicate that a large proportion of the metals is adsorbed externally (Smock 1983). In the present study, highly significant correlations were not observed for nymphs on any sampling day for any of the metals studied (except for Cd on day 50, $r^2 = 0.5$), *suggesting* that in our study the surface adsorption of these metals is quantitatively unimportant.

The inference of surface adsorption from body size – metal concentration relationships is based on the assumption that metabolic rates do not vary with body size, which is generally not the case (Zimmerman et al. 1975; Zimmerman and Wissing 1978). Secondly, it is assumed that the rate of surface adsorption is rapid compared with the time span of the larval or nymphal instar. This may not be the case for organisms which pass through many instars (such as mayflies like *Hexagenia*; Fink 1980) and/or where metal association with the body surface occurs by continuous adsorption. The need for this second assumption can be circumvented if the age of the nymphal skin (i.e. time since the previous moult) and not the size of the insect is used in such comparisons.

In the present experiments, the extent of iron oxide deposition on the body surfaces of *Hexagenia* was used as an index of nymphal skin age. These deposits were partially removed prior to dissection; however, removal was not complete, since a strong correlation was observed between the concentration of Fe in and on the body after dissection (data not shown) and a visual estimate of the extent of iron oxide coverage prior to dissection ($r^2 = 0.64$; visual scale from 1, no deposit, to 4, heavy deposit). There was little or no correlation between the visual scale and concentrations of Cd and Zn associated with the body ($r^2 = 0.16$ and 0.01, respectively), suggesting, as concluded above, that only minor proportions of these metals are adsorbed on the body surface. However, a strong positive relationship *was* observed for Pb (Fig. 7). Consistent with these trends, body Fe concentrations were highly correlated with those of Pb ($r^2 = 0.7$) but not Cd and Zn ($r^2 = 0.01$ for both).

These results suggest that much of the body Pb was located externally, in association with iron oxides, a fact which was not apparent when body size rather than an index of skin age was considered. Consequently, much of the variability among replicate measures of Pb (see Fig. 4 and 6 and Table 1) can be related to differences among nymphs in the time since their previous moult, i.e. the age of their skin. The differences observed among metals are consistent with the fact that Pb is more strongly bound to iron oxyhydroxides, which characterize the deposits, than are Cd and Zn (Leckie et al. 1983). These results suggest that Pb in sediments may have little direct toxic effect on *Hexagenia*; however, indirect effects of Pb on the sediment microflora consumed by *Hexagenia*, or on its predators, could be important.

Conclusions

A model based on first-order kinetics was adequate to describe the exchange of Cd, Pb, and Zn between nymphs of *H. rigida* and their surroundings. The exchange rates measured suggest that aquatic insects such as *Hexagenia* may be useful in monitoring changes over weeks or months. Since Cd and Zn appear to be taken up largely by the gut from sediment consumed as food, concentrations of these metals in *Hexagenia* would most likely reflect those in the sediment. In contrast, Pb associated with nymphs is largely bound with iron oxides adhering to their body surface. Given the fairly rapid exchange rates measured for Cd, depuration of nymphs prior to Cd analysis should be limited to a few days.

Acknowledgements

The authors acknowledge funding from the Ministère des Relations internationales du Québec (for travel assistance) and the Fonds pour la Formation de Chercheurs et l'Aide à la Recherche du Québec (postdoctoral fellowship to L. Hare) and research funding from the Natural Sciences and Engineering Research Council of Canada. Constructive comments on the use and measurement of radioisotopes were provided by R. Carignan and G. Kennedy. The latter also assisted in determining counter efficiencies. Technical assistance was provided by R. Beauchemin, R. Rodrigue, and B. Veilleux. The manuscript benefited from the comments of U. Borgmann, R. Carignan, C. Levings, and S. Micallef.

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Detection of Intraspecific DNA Sequence Variation in the Mitochondrial Cytochrome *b* Gene of Atlantic Cod (*Gadus morhua*) by the Polymerase Chain Reaction[1]

Steven M. Carr and H. Dawn Marshall

Genetics, Evolution, and Molecular Systematics Laboratory, Department of Biology, Memorial University of Newfoundland, St. John's, Nfld. A1B 3X9, Canada

Carr, S. M., and H. D. Marshall. 1991. Detection of intraspecific DNA sequence variation in the mitochondrial cytochrome *b* gene of Atlantic cod (*Gadus morhua*) by the polymerase chain reaction. Can. J. Fish. Aquat. Sci. 48: 48–52.

We determined the DNA sequence of a portion of the mitochondrial cytochrome *b* gene for 55 Atlantic cod (*Gadus morhua*) from Norway and from 10 locations within the Northern Cod complex and adjacent stocks off Newfoundland. DNA was prepared for sequencing by the polymerase chain reaction (PCR). Eleven variable nucleotide positions within a 298 base region defined 12 genotypes. Genotype proportions differed significantly between Newfoundland and Norwegian populations: the majority genotype among Newfoundland populations was present in a minority of Norwegian cod. Newfoundland cod showed less genotypic diversity than those from the eastern Atlantic: nine genotypes were found among all 10 Newfoundland populations, as compared with seven genotypes within the single Norwegian population. An exception was an overwintering, inshore Newfoundland population that showed four genotypes among five fish. As in other vertebrates, third position synonymous transitions predominate over other types of nucleotide changes. However, two amino acid replacement substitutions occur among cod, and the ratio of purine transitions to pyrimidine transitions is significantly higher than in other species. The existence of DNA sequence polymorphism permits the various hypotheses of the distribution and differentiation of Newfoundland cod stocks to be tested, and points to the utility of PCR technology in fishery genetics.

Nous avons déterminé la séquence de l'ADN d'une portion du gène mitochondrial codant le cytochrome *b* chez 55 morues franches (*Gadus morhua*) de Norvège et de 10 endroits se trouvant dans le secteur du complexe de la morue du nord et de stocks adjacents du large des côtes de Terre-Neuve. Pour les fins de l'analyse séquentielle, l'ADN a été préparé au moyen de la réaction en chaîne à la polymérase. Onze positions de nucléotides différentes ont été repérées dans un segment de 298 bases, soit 12 génotypes. Les proportions des génotypes variaient de façon significative entre les populations de Terre-Neuve et de Norvège: le génotype le plus fréquent dans les populations de Terre-Neuve était minoritaire parmi les populations norvégiennes. La diversité génotypique des morues de Terre-Neuve était inférieure à celle des morues de l'est de l'Atlantique: les 10 populations de Terre-Neuve comptaient neuf génotypes tandis que l'unique population de Norvège en comptait sept. Une population côtière hivernante de Terre-Neuve faisant cependant exception avec quatre génotypes observés sur cinq poissons. Comme chez les autres vertébrés, les transitions synonymes en troisième position étaient les modifications nucléotidiques les plus fréquentes. Cependant, des substitutions de deux acides aminés existent chez la morue, et la proportion des transitions des nucléotides pyrimidiques aux transitions des nucléotides puriques est significativement supérieure à celle observée chez d'autres espèces. L'existence d'un polymorphisme dans les séquences d'ADN de la morue permet de mettre à l'épreuve les différentes hypothèses sur la distribution et la différenciation des stocks de morue de Terre-Neuve et a de quoi susciter un intérêt pour l'utilisation de la technique de la réaction en chaîne à la polymérase dans le domaine de la génétique appliquée aux pêches.

Received December 18, 1989
Accepted July 23, 1990
(JA409)

Reçu le 18 décembre 1989
Accepté le 23 juillet 1990

Atlantic cod (*Gadus morhua*) in the western North Atlantic exist as several biologically distinct stocks (Templeman 1962). Cod in Northwest Atlantic Fisheries Organization (NAFO) divisions 2J3KL northeast of insular Newfoundland, referred to as the Northern Cod complex, have been managed as a single stock since 1973. The degree to which cod from the various banks, inshore, and offshore subcomponents within 2J3KL represent separate stocks is the subject of an ongoing debate with important fishery management implications (Lear 1984; Keats et al. 1986; Lear et al. 1986; Harris 1990).

Extensive protein electrophoretic data on genetic variation applicable to problems of stock discrimination in cod are available (Jamieson 1975; Cross and Payne 1978; Mork et al. 1985; Grant et al. 1987; Grant and Ståhl 1988a, 1988b): such data show little variation attributable to stock differences in the western Atlantic. An alternate genetic system, mitochondrial DNA (mtDNA), has in the last 10 yr found wide use in the study of local populations of many species (Wilson et al. 1985), including the definition of stocks in other fish species

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[1] The nucleotide sequence data reported in this paper have been submitted to GenBank and assigned the accession numbers M57651–57662.

(Gyllensten and Wilson 1987; Bickham et al. 1989). Conventional analysis of mtDNA has typically relied on physical purification of the molecule from individuals, cleavage with a series of Type II restriction endonucleases, and inspection of the resulting DNA fragment patterns for characteristic restriction fragment length polymorphisms (RFLPs). Smith et al. (1989) used this approach and reported no variation among 16 cod from the Grand Banks of Newfoundland, and RFLP variants in two fish from the North Sea, based on 21 mtDNA restriction fragments representing less than 0.8% of the genome. Johansen et al. (1990) reported a single RFLP among 21 Norwegian cod examined with eight restriction endonucleases.

The alternative to RFLP analysis, direct sequence determination, has until quite recently required molecular cloning: Johansen et al. (1990) recently determined the sequence of two thirds of the cod mitochondrial genome by this approach, and Beckenbach et al. (1990) compared a 2214 base pair portion of the same genome among six rainbow trout (*Oncorhynchus mykiss*). Cloning is, however, a technically demanding, laborious process that has precluded analysis of the large numbers of individuals required in population studies. This limitation has now been overcome by the advent of a new biotechnology, gene amplification by means of the polymerase chain reaction (PCR) (Saiki et al. 1988). Specific gene segments can be enzymatically amplified from a crude cell extract in sufficient quantities for direct sequencing. By appropriate choice of gene segments, it is possible to study DNA sequence variation among individuals, local populations, or species (Kocher et al. 1989; Vigilant et al. 1989).

In our ongoing study of cod, we wish to find genetic markers that can identify discrete components of the Newfoundland cod fishery, with a view towards improved management of this resource. Comparison of Newfoundland cod with those from the eastern Atlantic allows us to gauge the extent of intraspecific genetic differentiation. We present here results of our preliminary study of DNA sequence polymorphism in the mitochondrial cytochrome *b* region. This paper represents the first application of PCR technology to a fishery management question.

Materials and Methods

Cod from the western Atlantic were collected by personnel of the Department of Fisheries and Oceans, the Marine Sciences Research Laboratory, Memorial University, and local fishermen. Norwegian cod were supplied by R. Barrett, University of Tromsø. Localities, date of collection, and sample sizes are listed in Table 1.

Mitochondrial DNA was isolated or purified by either of two methods. First, cleared SDS detergent lysates of mitochondrial fractions from fresh or frozen (−20 or −70°C) cod hearts were subjected to ultracentrifugation in cesium chloride/propidium iodide gradients to obtain highly purified mtDNA (Carr and Griffith 1987). mtDNA was also amplified directly from the cleared lysates (steps 1–9 in the above procedure) without ultracentrifugation, and with NaCl substituted for CsCl in the final salt precipitation step.

We used as amplification and sequencing primers the following oligonucleotides, which correspond to highly conserved cytochrome *b* sequences identified by Kocher et al. (1989):

5′-ccatccaacatctcagcatgatgaaa-3′ (heavy-strand primer)
5′-gcccctcagaatgatatttgtcctca-3′ (light-strand primer).

TABLE 1. Origins of cod used in this study.

Location	*N*	NAFO Division	Date of collection
Tromsø, Norway	15	—	July 1989
Grey Island Shelf	7	3K	June 1988
Fogo Island[a]	5	3K	December 1989
Conception Bay	2	3L	July 1988
Flatrock[a]	1	3L	June 1989
Gull Island	2	3L	July 1988
Placentia Bay	2	3Ps	July 1988
Northern Grand Banks	4	3L	June 1988
Île aux Morts[a]	7	3Pn	January 1989
St. Pierre Bank	6	3Ps	January 1989
47°08′N, 55°07′W	3	3Ps	January 1989
47°00′N, 50°16′W	1	3O	January 1989
	—		
Total	55		

[a] Inshore fishery.

These primers amplify a 359 base pair region, which represents about 2% of the 16.5 kilobase cod mtDNA genome (Johansen et al. 1990). The primers were prepared on a Milligen oligonucleotide synthesizer in the DNA analysis facility at Memorial University.

Double-stranded (symmetric) PCR amplifications were carried out in 25-μL reactions containing 67 mM Tris (pH 8.8 at 25°C), 10 mM 2-mercaptoethanol, 2 mM $MgCl_2$ (all Sigma), 200 μM each of dATP, dCTP, dGTP, and dTTP (Pharmacia or Boehringer-Mannheim), 400 nM each of the heavy- and light-strand primers (10 pmol each per reaction), and 1 unit of Amplitaq polymerase (Perkin-Elmer Cetus). To this mixture was added 1 μL of the DNA preparation to be amplified, either purified mtDNA or the cleared lysate. The DNA was amplified in a Perkin-Elmer Cetus Thermal Cycler on the following step-cycle profile: strand denaturation at 92°C for 45 s, primer annealing at 50°C for 45 s, and primer extension at 72°C for 90 s, repeated for 30 cycles. Preliminary denaturation at 95°C for 5 min before the first cycle improved product yield in some cases.

Electrophoresis of a 10-μL portion of the amplification product was done for 1 h at 100 V in a 2% NuSieve gel (FMC) in Tris–acetate buffer (pH 7.4) containing ethidium bromide (1 μg/mL). DNA fragments were examined with 302-nm UV illumination. A small portion of each 359 base pair product was removed, added to 100 μL of H_2O, and remelted at 65°C for 10 min. Single-stranded (asymmetric) amplification was carried out on 1–2 μL of the remelted material under the same conditions as above, except that one primer was diluted 1:100 (final concentration 4 nM, 0.4 pmol added per reaction) and the total reaction volume was increased to 100 μL. (We typically diluted the light-strand primer, so as to obtain the light strand as the single-strand product; asymmetric amplification of the heavy strand was not routinely successful.)

The single-stranded DNA was desalted on a Centricon-30 ultrafiltration unit (Amicon) or an Ultrafree UFC-3 cartridge (Millipore). Single-stranded DNA sequencing reactions were prepared with Sequenase kits (version 2.0: U.S. Biochemical) and α-^{35}S-dATP (New England Nuclear) on 7 μL of the filter retentate, according to the manufacturer's directions; the label mix was diluted 1:50, which permits the sequence to be read immediately after the primer. Sequences were separated in 40-cm 6% polyacrylamide (19:1 BIS), 7 M urea gels. Electrophoresis was done at 30 W constant power (approximately 1600 V) for either 1–1.5 or 4–5 h to obtain the 5′ and 3′ ends of the sequence, respectively. The gels were fixed in 5% methanol/5% acetic acid, dried onto filter paper, and autoradiographed with Kodak AR or RP film.

Sequences were analyzed and prepared for publication with the help of the ESEE program (E. Cabot, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6). All sequences are given as their coding strand equivalents. Sequence variants were confirmed by resequencing of reamplified products from the extracted DNA.

Results and Discussion

Figure 1 shows the sequence of a 298 base portion of the coding strand of the most common cod cytochrome *b* sequence, along with the inferred amino acid sequence. Eleven nucleotide positions in this region vary among cod: the variable sites define 12 distinct genotypes (Table 2).

The distribution of these genotypes differs between the western and eastern Atlantic. Genotype proportions in the two areas are statistically differentiable: the frequency of the most common genotype (A in Table 2) is significantly smaller in the Norwegian population (27%) than in Newfoundland cod (80%) ($p << 0.01$, Fisher's exact test). Genotypic diversity is also lower in the western Atlantic. Among the 15 fish from the single Norwegian population, seven genotypes were found, four of these in at least two individuals each. In contrast, examination of the 40 fish from 10 Newfoundland populations revealed a total of nine genotypes; except for the common type, none was found in more than a single individual. Genotypic diversity can be quantified by the nucleon diversity index h of Nei and Tajima (1981): $h = (1 - \Sigma(x^2))(n)/(n-1)$, where x is the proportion of each genotype and n is the total number of individuals. By this measure, the single Norwegian population is substantially more variable ($h = 0.88$) than all Newfoundland populations combined ($h = 0.36$). An exception to this pattern is the Fogo Island sample, an overwintering, inshore component of the 2J3KL complex, which, with four genotypes among five fish ($h = 0.90$), was the most variable Newfoundland population.

The pattern of nucleotide substitution in the mitochondrial cytochrome *b* gene in cod differs from that of other vertebrate species studied. Nine of the 11 observed substitutions are synonymous substitutions at third positions, eight of which are transitions. This is similar to the pattern among closely related individuals of other vertebrate species, where transitions greatly outnumber transversions (Kocher et al. 1989). The same is true in a different portion of the mitochondrial genome of rainbow trout (Beckenbach et al. 1990). In cod, silent purine and pyrimidine transitions are equally frequent (4 versus 4). In rainbow trout, Beckenbach et al. (1990) found an excess of purine transitions (8 versus 4). In deer of the genus *Odocoileus*, however, 22 of 24 synonymous substitutions among individuals are pyrimidine transitions (S. M. Carr and G. A. Hughes, submitted). Both mammal/fish comparisons are significantly different ($p < 0.05$, Fisher's exact test). The remaining two substitutions in cod result in amino acid replacements: an a → g second position transition at nucleotide 80 replaces glutamic acid with glycine in genotype F, and a g → a first position transition at nucleotide 100 replaces valine with isoleucine in genotype L (Fig. 1). Neither replacement is considered radical (Grantham 1974). Replacement substitutions in this gene are unusual in closely related individuals of other vertebrate species (Kocher et al. 1989): in deer, no replacement substitutions were observed either within or between species (S. M. Carr and G. A. Hughes, submitted). (The difference is, however, not significant.) Beckenbach et al. (1990) found one replacement substitution among six trout. These differences may indicate varying patterns of mutation, fixation, and/or selection between bony fish and mammals.

Documentation of genetic variation and modification of management practices so as to preserve or restore genetic diversity are recognized goals of successful fishery management (Nelson

G	S	L	L	G	L	C	L	I	T	Q	L	L	T	G	15
ggc	tct	ctt	cta	ggc	ctt	tgc	tta	att	act	caa	ctt	cta	aca	gga	45
L	F	L	A	M	H	Y	T	S	D	I	E*	T	A	F	30
cta	ttt	cta	gcc	ata	cac	tat	acc	tca	gac	atc	gag	aca	gcc	ttc	90
						*		*			**				
S	S	V	V*	H	I	C	R	D	V	N	Y	G	W	L	45
tca	tcc	gta	gtc	cac	atc	tgt	cgt	gat	gta	aac	tac	ggc	tga	cta	135
			* *	*					*						
I	R	N	M	H	A	N	G	A	S	F	F	F	I	C	60
att	cgg	aat	ata	cat	gct	aat	ggt	gcc	tct	ttc	ttt	ttc	att	tgt	180
L	Y	M	H	I	A	R	G	L	Y	Y	G	S	Y	L	75
ctt	tat	atg	cac	att	gcc	cga	ggt	ctc	tat	tat	ggt	tcc	tat	ctt	225
						*									
F	V	E	T	W	N	I	G	V	V	L	F	L	L	V	90
ttt	gta	gag	aca	tga	aac	atc	ggg	gtt	gtc	ctt	ttc	ctt	tta	gta	270
					*		*								
M	M	T	S	F	V	G	Y	V							99
ata	ata	acc	tct	ttc	gta	ggt	tat	gtc	c						298

FIG. 1. Sequence of a 298 base region of the mitochondrial cytochrome *b* gene from cod (*Gadus morhua*). The sequence of genotype A in Table 2 is given: variable nucleotides and amino acids are marked with an asterisk (see text). The inferred amino acid sequence is indicated.

TABLE 2. Distribution of DNA sequence variation among populations of *Gadus morhua*. Sequences are identical to genotype A unless otherwise indicated. All 3K variants are found in the Fogo Island sample, except genotype H which occurs in the Grey Island Shelf sample.

	Nucleotide position in Fig. 1											Distribution						
Genotype	66	72	80	81	100	102	105	120	201	243	249	Nor	3K	3L	3O	3Pn	3Ps	*N*
A	t	a	a	g	g	c	c	a	a	c	g	4	8	9	1	6	8	36
B	·	·	·	·	·	·	·	g	·	·	·	2	—	—	—	—	—	2
C	·	·	·	·	·	·	·	·	·	t	·	1	1	—	—	—	—	2
D	·	·	·	a	·	·	·	·	·	t	·	1	—	—	—	—	—	1
E	c	·	·	·	·	·	·	·	·	·	·	3	—	—	—	—	1	4
F	c	·	g	·	·	·	·	·	·	·	·	1	—	—	—	—	—	1
G	·	·	·	·	·	·	·	·	·	·	t	3	1	—	—	—	—	4
H	·	·	·	·	·	·	t	·	·	·	·	—	1	—	—	—	—	1
I	·	·	·	·	·	t	·	g	·	·	·	—	—	—	—	1	—	1
J	·	·	·	a	·	·	·	·	g	t	·	—	—	—	—	—	1	1
K	·	g	·	·	·	·	·	·	·	·	·	—	1	—	—	—	—	1
L	·	·	·	·	a	·	·	·	·	·	·	—	—	—	—	—	1	1

and Soulé 1987). The existence of identifiable mtDNA sequence polymorphism in Newfoundland cod makes the various hypotheses of stock differentiation and discrete inshore breeding populations testable. Even with the relatively small samples sizes used here, it has been possible to differentiate transatlantic stocks. NAFO divisions 3Pn, 3Ps, and 3K, which contain banks that are believed to correspond to separate spawning areas (Templeman 1962; May 1966; Lear 1984), all contain variant genotypes at low frequency; one inshore population within 3K contains several such genotypes. The putative genetic and/or ecological distinctness of such stocks, particularly those of the inshore, is the subject of ongoing management interest (Pinhorn 1984; Keats et al. 1986; Lear et al. 1986; Harris 1990). More extensive variation is expected within more rapidly evolving portions of the mtDNA genome such as the D-loop control region (Brown 1983), which may be the region of choice for studies of intraspecific polymorphism (Vigilant et al. 1989). It remains to be seen whether these or other genetic markers can, with larger sample sizes, be used to differentiate spawning populations with statistical confidence (S. M. Carr and H. D. Marshall, work in progress).

The low genotypic diversity of Northern Cod populations is reminiscent of the pattern seen with allozymes (Cross and Payne 1978; Mork et al. 1985). Whether this pattern is general among cod stocks in the western Atlantic, and if so its causes, are questions for further investigation. The long-term consequences of absence of genetic diversity in fishery stocks are well known (Nelson and Soulé 1987). Reduced effective population size and consequent loss of heterogeneity in cod could result from historical factors such as Pleistocene glaciations (Cross and Payne 1978), bottlenecks at the time of population origin (Grant and Ståhl 1988a, 1988b), homogenizing effects of gene flow (Smith et al. 1989), and/or fishing patterns (Lear et al. 1986), among other possibilities.

Sequence variation observed in PCR studies of natural populations may be authentic polymorphism, or may be an artifact introduced by errors in the PCR process itself. The *Taq* polymerase used in PCR amplification is known to have a single-base substitution error rate of about 1 in 9000, due in part to the absence of $3' \rightarrow 5'$ exonuclease proofreading activity; heat damage to the template DNA and degradation of the *Taq* enzyme may also occur during repeated cycling to high temperature (Tindall and Kunkel 1988). These may result in artifactual sequence variation if replication errors occur in early rounds of amplification. Otherwise, products with induced mutations contribute relatively small proportions to the total product, so that direct sequencing yields a consensus pattern corresponding to the genomic sequence (Saiki et al. 1988). Amplification and sequencing of both complementary strands can corroborate the fidelity of reciprocal asymmetric amplifications, but cannot detect errors introduced in the initial symmetric amplification. The authenticity of sequence variants can only be verified by reamplifying and resequencing the same strand from the original DNA source, as was done here. Thermal effects can also be minimized by reducing the time of the denaturation step, and reducing the number of thermal cycles.

The PCR procedure offers several practical advantages over conventional restriction endonuclease methods for the analysis of intraspecific variation. The quantity of tissue and the quality of its preservation are much less problematic. Less than 100 mg of tissue was routinely used. Equally good double- and single-stranded DNA amplifications and sequencing results were obtained from purified or crude mtDNA preparations, from material poorly or not quickly frozen, from tissue held at −20°C for up to 2 yr, and from tissue that had been repeatedly frozen and thawed. These considerations are especially important when material is collected in the field. The results presented here also indicate that the method is more sensitive to subtle variation within populations. This study analyzed about 2% of the cod mitochondrial genome, more than twice as much as previous RFLP analyses of the same genome (Smith et al. 1989; Johansen et al. 1990), and has revealed a degree of intraspecific polymorphism that the previous studies, on populations of similar origin, did not. Technically, our experience is that direct sequence analysis of DNA amplified by PCR is simpler and more rapid than conventional restriction analysis of purified mtDNA. The limiting factor in this genetic assay is the need for collection, alignment, and detailed comparison of individual sequences by hand and eye. Automation of sequence acquisition and data handling (Landegren et al. 1988; Wilson et al. 1990) may be especially useful where homologous regions of a few hundred base pairs are to be compared repeatedly amongst many individuals. Such technology, in conjunction with PCR amplification of DNA as performed in this study, may be well suited for the genetic analysis of fish stocks.

Acknowledgements

This research is supported by the Canadian Centre for Fisheries Innovation, Department of Fisheries and Oceans Science Subvention awards and Natural Sciences and Engineering Research Council of Canada (NSERC) Operating and President's grants to S.M.C., an NSERC Equipment grant to I. R. Ball, S.M.C., D. J. Innes, and P. G. Hempstead, an NSERC Equipment grant to W. S. Davidson and S.M.C., and an NSERC summer student fellowship to H.D.M. We thank W. S. Davidson, G. A. Hughes, D. J. Innes, A. Meyer, P. Pepin, G. Rose, A. C Wilson, and two anonymous reviewers for discussion and critical comment. We thank personnel of the Department of Fisheries and Oceans, St. John's, Nfld., R. Barrett, D. Clark, R. Payne, J. Wroblewski, and many Newfoundland fishermen for cooperation in sample collection. The courtesy of members of the A. C. Wilson laboratory for assistance with early experiments and T. D. Kocher for providing primer sequences in advance of publication is also gratefully acknowledged.

NOTE ADDED IN PROOF:

There are to our knowledge no reported cases of artifactual sequence polymorphism in natural populations produced by the PCR process; PCR has, however, identified such artifacts in sequences obtained by cloning (Pääbo, S., and A. C. Wilson. 1988. Polymerase chain reaction reveals cloning artifacts. Nature (Lond.) 334: 387–388).

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Rheotactic Differentiation between Fluvial and Lacustrine Populations of Arctic Grayling (*Thymallus arcticus*), and Implications for the Only Remaining Indigenous Population of Fluvial "Montana Grayling"

Calvin M. Kaya

Biology Department, Montana State University, Bozeman, MT 59717, USA

Kaya, C. M. 1991. Rheotactic differentiation between fluvial and lacustrine populations of Arctic grayling (*Thymallus arcticus*), and implications for the only remaining indigenous population of fluvial "Montana grayling." Can. J. Fish. Aquat. Sci. 48: 53–59.

Rheotactic behavior of young Arctic grayling (*Thymallus arcticus*) from fluvial (Big Hole River) and lacustrine (Red Rocks Lake) populations in Montana was assessed in an artificial stream to see if fluvial grayling are adapted to life-long residence in streams by having an innately greater tendency to hold position and lesser tendency to go downstream. Responses of young tested at 0–10 d post-swimup contradicted the hypothesis; the fluvial grayling had strong downstream responses similar to or greater than those of the lacustrine grayling. When tested 18–31 d post-swimup, however, rheotactic responses of the fluvial and lacustrine grayling were consistent with the hypothesis, at three light intensities (full and dim lighting and darkness). Rheotactic differences were even greater in trials at 47–72 d post-swimup (conducted only under full lighting). Big Hole River grayling appear to be adapted to permanent stream residence. Such adaptation reinforces the importance of conserving this last indigenous fluvial population of the geographically disjunct, genetically identifiable "Montana grayling."

Le comportement rhéotaxique de jeunes ombres arctiques (*Thymallus arcticus*) de rivière et de lac (rivière Big Hole et lac Red Rocks, au Montana) a été étudié dans un cours d'eau artificiel afin de déterminer si le fait que les ombres de rivière passent toute leur vie en rivière est le fruit d'une adaptation se traduisant par une tendance innée plus forte à rester en rivière qu'à se diriger vers l'aval. Les essais effectués avec de jeunes ombres 0 à 10 jours après qu'ils aient été en mesure de nager contredisent cette hypothèse : les ombres de rivière se dirigeaient vers l'aval tout autant ou même davantage que les ombres de lac. Cependant, 18 à 31 jours après qu'ils aient commencer à nager, les essais menés à trois intensités lumineuses différentes (pleine lumière, lumière atténuée et obscurité) venaient corroborer l'hypothèse. À 47 à 72 jours, les différences dans le comportement rhéotaxique étaient encore plus marquées (essais en pleine lumière seulement). Les ombres de la rivière Big Hole semblent adaptés à séjourner de façon permanente en rivière. Une telle adaptation rend encore plus impérative la nécessité de conserver cette dernière population indigène de rivière de l' "ombre du Montana," ombre géographiquement isolé ayant des caractéristiques génétiques propres.

Received December 20, 1989
Accepted July 23, 1990
(JA413)

Reçu le 20 décembre 1989
Accepté le 23 juillet 1990

Intraspecific differences in directional responses to water current (rheotaxis) of young salmonids are well known (reviewed by Thorpe 1981; Godin 1981; Northcote 1984) and appear to be important adaptations to differing patterns of migration and habitat utilization among populations (e.g. anadromy versus nonanadromy, or lake inlet versus outlet spawning). Evidence that intraspecific rheotactic differences in salmonids are genetically based has come from studies on *Oncorhynchus* and *Salmo* (Taylor and Larkin 1986; and reviews by Godin 1981; Northcote 1984) and on Arctic grayling (*Thymallus arcticus*) (Kaya 1989). Such innate behavioral variation contributes to genetic diversity within species and to the ability of a species to continue occupying different habitats within its distributional range (MacLean and Evans 1981; Swain and Holtby 1989).

Rheotactic adaptation could be important to the existence of Arctic grayling in streams, including the only indigenous fluvial population remaining south of Canada and Alaska. Once common in the upper Missouri River and its tributaries within the present State of Montana, USA, these southernmost fluvial grayling have been reduced to one remnant, indigenous population inhabiting the Big Hole River (Liknes and Gould 1987). Arctic grayling in Montana (hereafter referred to as "Montana grayling") are genetically distinguishable from those in Canada and Alaska (Lynch and Vyse 1979; Everett and Allendorf 1985). The substantial reduction in distribution and apparent continuing decline of fluvial Montana grayling has resulted in their designation as fish of "special concern" by the Endangered Species Committee of the American Fisheries Society (Deacon et al. 1979; Johnson 1987).

There have been no previous experimental studies to determine whether young from fluvial (spawning and living permanently within streams) and lacustrine (living in lakes and entering streams to spawn) populations of a given species differ innately in their rheotactic responses. The possibility of such behavioral differentiation is supported by a recent study on anadromous chinook salmon (*Oncorhynchus tshawytscha*) which demonstrated that "stream-type" young that rear in a stream before migrating to the sea had stronger positive response to current than did "ocean-type" young that rear in an estuary (Taylor and Larkin 1986). If fluvial Montana grayling differ from lacustrine Montana grayling (still common as indigenous and introduced populations) in possessing adaptations enabling them to reside permanently in streams, then their conservation would be essential for the continued presence of these fish in streams. I tested the hypothesis that young fluvial Montana grayling differ from lacustrine grayling in having a greater tendency to hold position and lesser tendency to go downstream in water current, and that such differences are genetically controlled.

Methods

Young fish originated from fertilized eggs produced in May 1988 from fluvial grayling of the Big Hole River and from the inlet-spawning, lacustrine grayling of Red Rocks Lake. Both are in the drainage of the upper Missouri River in southwestern Montana and contain native populations of Arctic grayling (Nelson 1954; Liknes and Gould 1987). Ripe adults were captured by electrofishing and were stripped of gametes to produce fertilized eggs on location. Despite an intensive effort, only six ripe females were taken from the Big Hole River, and the eggs from each were fertilized with sperm from one or two different males, for a total parental pool of 15 fish. The collection from Red Rocks Lake was part of a hatchery project by the Fish Cultural Development Center (Bozeman, MT) of the U.S. Fish and Wildlife Service and about 40 of each sex were used to produce a large number of fertilized eggs. The fertilized eggs were placed in jars and transported in insulated, chilled containers to the Fish Cultural Development Center. Eggs from a given location were mixed together and a subsample was taken of those from Red Rocks Lake to be treated identically to those from the Big Hole River.

Embryos and young from both sources were incubated and reared in 12 cuboidal, 90-L tanks located side by side in four adjacent rows and supplied from a common water source. Warm and cold spring water were mixed to control incubation and rearing temperatures, and flow rate through the rearing tanks was set to 4–5 $L\cdot min^{-1}$. The tanks received natural, indirect lighting from a nearby window, supplemented between about 0800 to 1700 with artificial overhead lighting. Embryos and young were incubated in screened trays floating in the tanks at 8.5–10°C until they hatched, absorbed their yolk sacs, and started to swim freely. The young were released into the tanks as they became free swimming and the rearing temperature was increased to 10–12°C. Hereafter, ages of the young grayling refer to days post-swimup, with day 0 the date when most (about 80–90%) from a given population had become free swimming. Rearing temperature was maintained at 10–12°C for about 6 wk and then at 15 ± 1.0°C until tests were completed on fish about 10 wk old. The young were fed commercial trout pellets of increasing particle size as the fish grew. Fertilized eggs were measured with an ocular micrometer and young with a ruler. Samples for measurement were taken by pouring eggs out of the incubating trays, or by sweeping through the rearing tanks with a net, until 50 specimens were obtained.

Young were initially distributed among three rearing tanks for each of the two populations, starting at approximately 2000 per tank. Within each population, fish for a given trial were taken from the same tank and tanks were sampled in rotating sequence so that densities in each tank were reduced at the same rate and fish from a different tank were tested first on different days. Tested fish from each population were returned to three other tanks for further rearing. When the supplies of untested young were exhausted (after 31 d post-swimup for fluvial fish and 27 d for lacustrine fish), the previously tested fish were redistributed among the six rearing tanks for each population and maintained until the final trials at 47–72 d. Fish were not retested and therefore were not exposed to flowing water until being placed in the artificial stream, with the exception that those tested at 47–72 d had been previously tested at 0–31 d post-swimup.

Responses to water current were tested in an artificial stream consisting of an oval, compartmentalized tank (Kaya 1989, his fig. 1). Water velocities over each notched baffle between tank compartments ranged from 6–7 $cm\cdot s^{-1}$ over the center to 3 $cm\cdot s^{-1}$ over the shallow margins. The maximum velocities were within the range (5–10 $cm\cdot s^{-1}$) selected by newly swimming young European grayling (*Thymallus thymallus*) (Scott 1985). The slowest water velocities were <1 $cm\cdot s^{-1}$ along the bottoms and corners within the compartments. Test temperatures were maintained at selected levels (10, 12–13, 15°C) with a mixture of warm and cold spring water, the same water in which the young were incubated and reared, at a flow rate of 20–25 $L\cdot min^{-1}$.

Four series of trials, each on a given age group of fish from both populations, were conducted under full illumination from twenty 40-W fluorescent lamps. Light intensity just beneath the water surface of the artificial stream ranged from 10.1 to 15.6 $\mu E\cdot m^{-2}\cdot s^{-1}$. Trials were conducted during natural daylight hours, with consecutive trials within a day starting from about 0630 to 1400. The young were tested at swimup (day 0) and at 9–10, 18–27, and 47–72 d post-swimup. Big Hole River grayling were, on average, smaller than those of Red Rocks Lake at all ages from swimup to the oldest tested (Table 1). For the last series of trials, Big Hole River young were tested at 60 d and Red Rocks Lake young were tested at 47 d, when they were similar in size to 60-d Big Hole River young (Table 1), and again at 72 d (because of other commitments I was unable to test Red Rocks Lake young at 60 d).

I started each trial by placing approximately 200 Big Hole River or Red Rocks Lake grayling into the release chamber. With most trials, I then left the room in which the test tank was located in order to minimize disturbance of the fish and avoid the possibility of affecting their responses. For a few trials I sat beneath one arm of the tank to observe the behavior and movement of fish in the opposite arm above; they seemed not to notice my presence during such observations. After 3 h, I determined the distribution of fish among the stream compartments. This test duration was selected on the basis of preliminary trials which indicated that this was a sufficient time for at least 70% of lacustrine grayling (2 wk old) to move out of the release chamber and into the upstream and downstream traps. At the end of the trial, fish within the intermediate compartments, in the traps, and in the release chamber were counted and removed.

Because downstream movement of young salmonids occurs predominantly in the evening or at night (Kruse 1959; Northcote 1962, 1969; McCart 1967; Brannon 1972), I also conducted trials under dim lighting and in darkness, on fish 25–31 d in age. Procedures were the same as for trials under full illumination, with two modifications. First, a transitional period of fading light was provided during which screens were placed to prevent the fish from leaving the release chamber. The over-

TABLE 1. Mean sizes of fertilized eggs, and of young at ages tested for rheotactic responses, of grayling from the Big Hole River and Red Rocks Lake. Data are mean ($n = 50$) diameters of eggs and total length of young (mm (SD in parentheses)).

		Post-swimup age of young				
Source	Eggs	0 (swimup)	20–21 d	40–44 d	60 d	72 d
Big Hole	3.4 (0.1)	9.6 (0.3)	18.2 (1.6)	30.2 (2.9)	46.6 (5.8)	
Red Rocks	4.2 (0.2)	10.8 (0.4)	21.2 (1.6)	44.6 (5.0)		60.2 (6.0)

head lamps were turned off 10 min after the young were placed into the release chamber, leaving the test tank illuminated by two 120-W incandescent floodlamps, one each over opposite ends of the test tank. Over the next 15 min the intensity of lighting from the floodlamps ($3.6\ \mu E \cdot m^{-2} \cdot s^{-1}$ in the center of the release chamber) was progressively reduced by an automated rheostat until the lamps were off, leaving the tank either under dim lighting ($0.03–0.05\ \mu E \cdot m^{-2} \cdot s^{-1}$) or in darkness. The screens were then removed to commence the test period. Second, these trials lasted 1 h instead of 3 h, because preliminary trials indicated that young grayling moved out of the release chamber and into upstream and downstream traps much faster in dim light or in darkness than under full illumination.

Upstream and downstream movements were compared by two methods. One method was the calculation of a ranked response which considered distance as well as direction moved during the trial. The compartments and traps were numbered sequentially in each direction from the release chamber (the end of the oval opposite from the inflow and outflow) from 1 for the immediately adjacent compartments to 5 for the farthest compartments, which were the upstream and downstream traps. The upstream and downstream scores for each trial were calculated by multiplying the number of fish in each compartment at the end of the trial by the compartment number, summing the products for each direction, and dividing each of the two sums by the total number of fish in the trial. The second method compared direction of movement — upstream, downstream, or remaining stationary within the release chamber — without considering the distance moved. The numbers of fish in intermediate compartments were combined with those within the traps to give the total "upstream response" or total "downstream response" as percentages of total number in the trial. The "stationary reponse" was the percentage remaining within the release chamber at the end of the trial.

Responses of fluvial and lacustrine grayling were compared for each series of trials through one-way analysis of variance (ANOVA). If ANOVA indicated a significant difference ($p < 0.05$), then the Newman–Keuls multiple comparisons test was used to identify differences among two or more groups (alpha = 0.01 and 0.05). Percentage responses were transformed (arcsin square root) for the statistical comparisons (Steel and Torrie 1960). Both methods of comparison, ranked responses and total percentage responses, produced nearly identical statistical comparisons between fluvial and lacustrine fish. For this reason and also because the tendency to remain stationary cannot be expressed as a response ranked according to distance moved, total percentage responses are presented in the results. Unless otherwise specified, significant differences indicate $p < 0.05$.

Results

Qualitative Behavioral Observations

Young grayling tested at 0–31 d post-swimup moved through the compartments as individuals, without any apparent interaction with others. By contrast, interactions among fish tested at 47–72 d post-swimup were highly variable, with some moving individually and others in groups of varying sizes. Younger (0–31 d) fish tended to spend most of their time within a compartment in the slowest water close to the bottom or corners, while older (47–72 d) fish tended to swim actively throughout each compartment. Fish moving downstream tended to swim over the baffles headfirst, rather than drifting backward, suggesting active movement rather than passive drift. These observations were made during trials under full and dim lighting and I could not determine whether their behavior in darkness was similar.

Responses under Full Illumination

Responses of fluvial and lacustrine grayling tested on the first day of swimup at the incubation temperature of 10°C were contrary to those hypothesized. Fluvial young had significantly greater mean downstream response and significantly weaker mean upstream response than did the lacustrine young (Table 2). Young from both populations had relatively high mean stationary responses which did not differ significantly. The relatively high percentages of fishes remaining stationary may have represented, at least in part, resting rather than rheotactic behavior. Grayling placed in the test tank as they first started swimming tended to spend long periods resting on the bottom between short periods of active swimming.

Rheotactic responses of young grayling from both populations were similar at 9–10 d post-swimup, with no significant differences between their mean upstream, downstream, or stationary responses (Table 2). Both fluvial and lacustrine grayling had similarly strong downstream responses and relatively weak upstream and stationary responses. Responses were similar at 10 and 12–13°C (Table 2).

Fluvial young tested at 19–21 d post-swimup (12°C) underwent changes in rheotactic responses with time of day (Table 3). In trials started at about 0630, their mean stationary response was significantly weaker and their mean upstream response significantly stronger than in trials started in midmorning and early afternoon. Consistent with their upstream responses, mean downstream response was stronger in the early morning than later in the day, but the differences were not significant (Table 3). By contrast, rheotactic responses of similar-age (18–22 d) lacustrine young changed slightly, but not significantly, with time of day (Table 3). Diel changes in rheotactic responses were not observed in grayling from either population tested in other series of trials in this study. Despite changes in responses with time of day in fluvial fish, they still had significantly weaker mean downstream response overall than did lacustrine young (Table 3). Fluvial fish also had significantly greater mean stationary response than did lacustrine fish in trials started both in midmorning and in the afternoon (Table 3).

TABLE 2. Rheotaxis of young grayling from fluvial (Big Hole River) and lacustrine inlet-spawning (Red Rocks Lake) populations tested in an artificial stream at different post-swimup ages, temperature, and light levels (full = 10.1–15.6 $\mu E \cdot m^{-2} \cdot s^{-1}$, dim = 0.03–0.05 $\mu E \cdot m^{-2} \cdot s^{-1}$); n = approximately 200 per trial. Within each column of upstream, downstream, and stationary responses for each series of trials (given age group and light intensity), mean percentages that do not share a common letter are significantly different: unshared lowercase letters indicate $p < 0.05$, unshared uppercase letters indicate $p < 0.01$ (ANOVA and Newman–Keuls multiple range test on arcsin square root transformations of percentage responses).

Origin	Trials	Total n	Age (d)	Test temp. (°C ± 0.2)	Mean % (SD) upstream	Mean % (SD) downstream	Mean % (SD) stationary
Day of swimup, full light							
Big Hole River	3	561	0	10.0	7.5 (2.9) a	43.0 (18.4) a	49.5 (21.0) a
Red Rocks Lake	4	787	0	10.0	24.6 (8.5) b	14.2 (7.8) b	61.2 (5.7) a
9–10 d post-swimup, full light							
Big Hole River	3	609	9	10.0	15.5 (5.0) c	79.5 (2.6) c	5.1 (2.7) b
Big Hole River	1	211	9	13.0	5.2 c	77.7 c	17.1 b
Red Rocks Lake	3	600	10	10.2	12.3 (1.8) c	77.0 (7.3) c	10.7 (6.6) b
Red Rocks Lake	3	604	9	12.0	10.3 (1.3) c	74.2 (11.3) c	15.6 (11.7) b
18–27 d post-swimup, full light							
Big Hole River	9	1806	19–21	12.2	25.1 (19.0) d	31.5 (11.3) D, d	43.4 (27.7) c
Big Hole River	1	200	27	15.4	16.0 d	37.0 D, d, E	47.0 c
Red Rocks Lake	10	2002	18–22	12.0	17.7 (10.5) d	61.1 (8.8) E, e	21.2 (14.7) c
47–72 d post-swimup, full light							
Big Hole River	3	600	60	15.0	9.2 (7.7) e	4.5 (2.2) F	86.3 (9.0) D
Red Rocks Lake	3	600	47	15.0	11.8 (1.4) e	75.3 (6.0) G, g	12.8 (4.5) E, e
Red Rocks Lake	3	600	72	15.0	16.3 (11.0) e	81.3 (13.2) G, g	2.3 (2.8) E, f
25–31 d post-swimup, dim light							
Big Hole River	6	1220	25–31	12.1	9.8 (4.4) f	22.6 (7.4) H	67.5 (11.5) g
Red Rocks Lake	3	600	27	12.0	7.2 (0.6) f	49.3 (0.6) I	43.5 (1.0) h
26–31 d post-swimup, darkness							
Big Hole River	4	799	26–31	12.2	3.6 (2.1) g	87.1 (5.8) j	9.2 (4.5) i
Red Rocks Lake	4	803	26–27	12.4	0.5 (0.4) h	97.4 (2.2) k	1.9 (2.1) j

TABLE 3. Effect of starting time of trials (Mountain Standard Time) on rheotaxis of young grayling from fluvial (Big Hole River) and lacustrine inlet-spawning (Red Rocks Lake) populations tested 18–22 d post-swimup. All trials conducted at 12.1 ± 0.3°C under full light intensity (10.1–15.6 $\mu E \cdot m^{-2} \cdot s^{-1}$); n = approximately 200 per trial. Within a given column of responses (upstream, downstream, stationary), means sharing a common letter are not significantly different ($p > 0.05$, Newman–Keuls multiple range test on arcsin square root transformations of percentage responses).

Start time ± 15 min	Trials	Total n	Mean % (SD) upstream	Mean % (SD) downstream	Mean % (SD) stationary
Big Hole River					
6:30 a.m.	3	601	48.9 (7.6) a	42.9 (6.3) c	16.5 (2.9) f
10:15 a.m.	3	603	16.4 (7.4) b	27.7 (4.7) d	48.4 (5.5) g
2:00 p.m.	3	602	10.0 (4.5) b	23.9 (12.2) d	54.7 (6.7) g
Red Rocks Lake					
6:30 a.m.	4	800	20.7 (6.8) b	68.1 (7.9) e	11.1 (4.3) f
10:15 a.m.	3	599	16.9 (18.7) b	54.4 (5.6) c,e	28.7 (21.0) f
2:00 p.m.	3	603	14.5 (6.4) b	58.4 (6.6) c,e	27.2 (12.8) f

For combined results at all times of the day, fluvial young had mean downstream response significantly weaker and mean stationary response stronger, although not significantly different, from that of lacustrine young (18–22 d post-swimup, 12°C, Table 2). The lack of statistically significant difference in mean stationary responses was probably related to the high variances produced by combining the dissimilar responses of fluvial fish tested in the early morning versus at other times of the day. Young trout (*Salmo trutta*) have been observed to avoid downstream movement both by remaining stationary and by going upstream (Elliott 1986); mean upstream plus stationary response of Big Hole River young for all times of the day combined was greater than that of lacustrine young at 18–22 d (ANOVA, $p < 0.001$).

Between 47 and 72 d post-swimup, differences in rheotactic behavior between fluvial and lacustrine fish were greater; 60-d fluvial grayling had much stronger mean stationary response and much weaker mean downstream response than did similar-sized lacustrine fish at 47 d, or larger lacustrine fish at 72 d post-swimup (Table 2). Responses of lacustrine young at 47 and 72 d post-swimup were generally not significantly different from each other, except for a small but significant difference between mean stationary responses. These older fish had been reared and tested at warmer temperatures (about 15°C) than the younger fish (reared at 10–12°C and tested at about 12°C). Very limited data from one trial on younger fluvial fish (27 d) at about 15°C (Table 2) suggest that responses at both 12 and 15°C are similar and that the greater differences between the older fluvial and lacustrine fish were not influenced by the higher test temperature.

Responses under Dim Lighting and in Darkness

Young grayling from the two populations also differed in rheotactic responses when tested under dim lighting (at 25–31 d) and in darkness (at 26–31 d). In dim lighting, fluvial fish had significantly greater mean stationary response and significantly weaker mean downstream response than did lacustrine fish (Table 2), but did not differ significantly in their mean upstream responses. Young grayling from both populations had strong downstream responses in darkness (Table 2); however, fluvial young still had significantly greater mean stationary response and significantly weaker mean downstream response than lacustrine young.

Discussion

While the ecological significance of differing responses to water current between young of fluvial and lacustrine populations seems apparent, experimental comparisons of their rheotaxis can be complicated if this behavior changes with age. If young of a lacustrine population have an initial period of residence in the spawning stream, then their rheotactic responses to test conditions during this period could be similar to those of young of a fluvial population. Conversely, if fluvial young disperse downstream from the immediate spawning areas after swimup, then their rheotactic responses during this period could be similar to those of migrating young of an inlet-spawning population. Initial downstream movement followed by position holding or upstream movement have been reported for sockeye salmon (*Oncorhynchus nerka*) emerging from spawning areas in the outlet stream or a tributary to the outlet of their nursery lake (McCart 1967; Brannon 1972).

Such behavioral changes with age may account for the seemingly contradictory responses of the grayling tested within the first 10 d after swimup, when fluvial young had strong downstream responses similar to or greater than those of lacustrine young. Nelson's (1954) observations indicate that young Red Rocks Lake grayling have an initial period of stream residence. He reported that grayling were abundant in the spawning creek for 2–3 wk after they first started emerging, but then apparently migrated downstream to Red Rocks Lake. Young grayling were not common near the mouth of the spawning creek until about 2 wk after their numbers peaked within the spawning areas a few kilometres upstream. On the other hand, young Big Hole River grayling appear to move initially downstream, both from spawning tributaries into the river (Liknes and Gould 1987) and from spawning riffles within the river into pools (Skaar 1989; Shepard and Oswald 1989).

By contrast, at an age of about 3 wk post-swimup, when in nature Red Rocks Lake young have moved or are moving into the lake (Nelson 1954), Big Hole River grayling had a significantly stronger tendency to hold position and a weaker tendency to move downstream. These differences in response between Big Hole River and Red Rocks Lake young became even greater when fish were tested at 47–72 d post-swimup. Such changes in rheotaxis with age may provide a possible explanation for results of studies in which experiments on early post-swimup salmonids did not confirm hypothesized rheotactic differences (e.g. Kelso et al. 1981; Taylor 1988).

My results also indicate that differences in downstream response were not due to size-related differences in swimming strength and ability to resist downstream displacement. Lacustrine grayling tested at about 3 wk or older post-swimup had a consistently greater tendency to move downstream than fluvial grayling, even though the large size of the lacustrine grayling should have given them an advantage in maintaining position in the water current (Heggenes and Traaen 1988). Lacustrine grayling also had a stronger tendency to move downstream as they got older despite their increasing size. These observations are in agreement with those of Brannon (1972), who concluded that size differences could not account for directional movement of young sockeye salmon, either within or between stocks.

The distinct pattern of change in rheotaxis with time of day by 19- to 21-d fluvial fish — weak stationary response early in the day, with similar movement upstream and downstream, followed by much stronger stationary response later in the morning and afternooon — was unexpected and is not easily explainable. One possibility is that this change in response may reflect diel activity patterns as, for example, movement either upstream or downstream early in the day to feeding areas or as a mechanism for dispersal within the stream. This possibility is supported by recent observations (McMichael 1990) that young grayling in the Big Hole River appear to move from pools to riffles early in the day.

Another possible explanation for this temporal change is that responses of fluvial fish may have been modified by olfactory cues. Young coho salmon (*Oncorhynchus kisutch*) may prefer water conditioned by the odor of fish from their own population (Quinn and Tolson 1986). Since water was recirculated within the test tank while concurrently being slowly replaced, odors from tested fish could accumulate as trials were conducted during the day, but be diluted overnight before the first trial in the morning. If odor affected the responses of young grayling in this series of trials, then interpretation of results would be modified: when not exposed to odors of fish from their respective

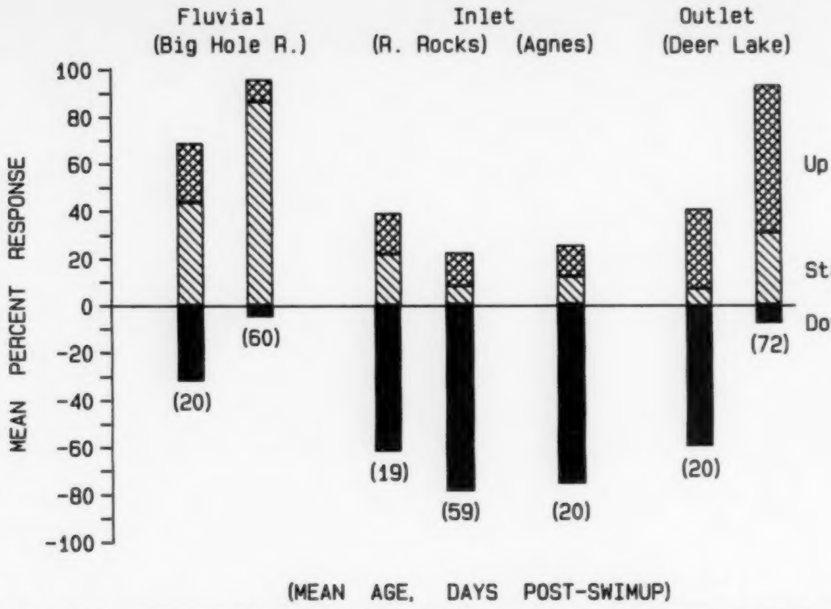

FIG. 1. Mean percentages of young fluvial (Big Hole River), lacustrine inlet-spawning (Red Rocks Lake, Lake Agnes), and lacustrine outlet-spawning (Deer Lake) grayling going upstream, downstream, and remaining stationary in an artificial stream when tested at about 3–10 wk post-swimup. All trials conducted under illumination of 10.1–15.6 $\mu E \cdot m^{-2} \cdot s^{-1}$, temperature of 12–15°C, and maximum water velocity of 6–7 $cm \cdot s^{-1}$; n = approximately 200 per trial. Numbers of trials and standard deviations for each mean response are given in Table 2 for Big Hole River and Red Rocks Lake fish. Data for Lake Agnes and Deer Lake fish are from a previous study (Kaya 1989): Lake Agnes (20 d, 5 trials), mean percentage upstream = 13.6 (SD 7.6), stationary = 11.5 (1.5), downstream = 75.2 (6.1); Deer Lake (20 d, 10 trials), upstream = 33.5 (3.5), stationary = 7.3 (3.3), downstream = 59.2 (4.9); Deer Lake (72 d, 3 trials), = 62.3 (6.4), stationary = 30.6 (7.1), downstream = 7.0 (1.7).

populations (first daily trials), fluvial fish had a stronger tendency to move upstream than did lacustrine fish; when exposed to their odors (subsequent daily trials), fluvial fish had a greater tendency to hold position in the current than did lacustrine fish; and with or without exposure to their odors, fluvial fish had a weaker tendency to swim downstream. If olfaction did affect their responses, the differences in rheotaxis between fluvial and lacustrine grayling in the presence of a recognized odor would still appear innate, and all three responses — greater tendency to remain stationary, greater tendency to swim upstream, or weaker tendency to move downstream — could serve to prevent downstream loss of the fluvial grayling. However, olfaction would not explain why this diel change in response was unique to fluvial grayling tested at this age.

Responses of fluvial fish in darkness did not appear to be consistent with behavioral adaptation for maintaining position in a stream. While the strong downstream responses of the lacustrine fish support previously cited observations that downstream migrations of young salmonids occur predominantly in the evening or at night, fluvial fish also had a strong, though significantly weaker, downstream response. It is possible that the downstream responses in darkness of young from both sources may have been promoted in part by experimental conditions. The artificial stream did not provide shallow areas or particulate substrate, within which young rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) and coho salmon have been reported to take shelter to help prevent their downstream displacement at night when visual cues are lacking (Hoar 1958; Northcote 1962). Or the downstream movement in darkness of the Big Hole River fish may reflect a diel shift in habitat use, for example, one in which the young may move downstream out of riffle areas and into pools at night.

The present observations of differing rheotactic responses between fluvial and lacustrine grayling about 3 wk or older in age, tested under full illumination at about 12–15°C, provide interesting comparisons with findings of a previous study (Kaya 1989) on similar-aged grayling of two other lacustrine populations, one inlet-spawning (Lake Agnes) and one outlet-spawning (Deer Lake), tested with the same procedures and apparatus. For this comparison (Fig. 1), both stationary and upstream responses are depicted as positive responses, since either would prevent downstream movement of fluvial young. Also, because of their similarity in responses, both older test groups of Red Rocks Lake fish (47 and 72 d) are combined into one older group with a mean age of 59 d. As younger fish averaging 19–20 d, young of both inlet-spawning populations (Red Rocks Lake and Lake Agnes) had very similar rheotactic responses, with strong downstream responses and weak upstream and stationary responses. Young of the fluvial (Big Hole River) population had the strongest stationary response, while those of the lacustrine, outlet-spawning (Deer Lake) population had the strongest upstream response among the four populations. These differences were even greater among older Big Hole River, Red Rocks Lake, and Deer Lake fish averaging 59–72 d (because of insufficient number of specimens, Lake Agnes grayling were not tested at a later age).

A behavioral tendency of the young to hold position in a water current could be as important an adaptation for permanent residence of a species in fluvial habitats as other characteristics that have been described for stream-resident salmonids (e.g. Taylor and Larkin 1986; Taylor 1988). While the present study addresses only one possible adaptation, the results support the hypothesis that grayling of the Big Hole River are behaviorally adapted for maintaining life-long residence in a stream. The genetic basis of such behavior is further supported by observations of intermediate rheotactic responses of hybrid young from cross-fertilizations between populations with contrasting migratory requirements in grayling (Kaya 1989) and other salmonids (Brannon 1972; Kelso and Northcote 1981).

Montana populations of Arctic grayling have been isolated for perhaps 75 000 to 100 000 yr (Lynch and Vyse 1979) and are electrophoretically distinguishable from Canadian and Alaskan populations (Lynch and Vyse 1979; Everett and Allendorf 1985). The fluvial, Big Hole River grayling are also electrophoretically distinguishable from lacustrine populations in Montana (Everett and Allendorf 1985). Grayling of the Big Hole River may thus represent a unique deme, a remnant, indigenous population of the geographically isolated, genetically identifiable "Montana grayling" that is adapted to life-long residence in a fluvial environment. Conservation of this declining population may be essential for the continued ability of these fish to live in streams.

Acknowledgments

This study was supported in part by grants from the Madison-Gallatin Chapter of Trout Unlimited to the author. Construction of the artificial stream was made possible by grants to Ray J. White from the Trout and Salmon Foundation, the Federation of Fly Fishers, the Montana Trout Foundation, and the Montana Department of Fish, Wildlife and Parks. Facilities, supplies, aid for incubation and rearing of embryos and young fish, and space and utilities for the experimental apparatus were provided by the Fish Cultural Development Center (U.S. Fish and Wildlife Service) in Bozeman, MT.

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Life History Changes in Eastern Mosquitofish (*Gambusia holbrooki*) Induced by Thermal Elevation

Gary K. Meffe

University of Georgia's Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29801, USA

Meffe, G. K. 1991. Life history changes in eastern mosquitofish (*Gambusia holbrooki*) induced by thermal elevation. Can. J. Fish. Aquat. Sci. 48: 60–66.

Much light can be shed on life history evolution through study of responses of organisms to chronic exposure to a novel or perturbed environment. To determine the influence of 28 yr of temporally unpredictable thermal elevation on their life history patterns, I sampled eastern mosquitofish (*Gambusia holbrooki*) from a thermally elevated (outflow from a nuclear reactor) and an ambient (farm pond) habitat in South Carolina every month for 2 yr. Fish from the artificially heated environment reproduced all year, had higher reproductive investments (higher clutch sizes and reproductive biomass), and smaller offspring than did fish from the ambient environment, which ceased reproduction from October through March, typical for natural populations of the region. Likely environmental factors responsible for these differences include unpredictable food resources, higher mortality from thermal death, and higher predation by fishes and birds in the heated waters. The extent to which these life history alterations are the result of adaptive genetic changes versus phenotypically plastic responses remains to be tested.

L'étude des réactions des organisms vivants exposés de façon chronique à un environnement nouveau ou perturbé peut nous renseigner grandement sur l'évolution de leur cycle vital. Afin de déterminer l'effet d'une élévation subite et durable (28 ans aujourd'hui) de la température sur le cycle vital de la gambusie *Gambusia holbrooki*, j'ai échantillonné mensuellement pendant 2 ans des individus de cette espèce d'une part dans un habitat dont la température est élevée (effluent d'un réacteur nucléaire) et, d'autre part, dans un habitat dont l'eau est à température ambiante (étang d'exploitation piscicole) dans la Caroline du sud. Les poissons du milieu chauffé artificiellement se reproduisaient à l'année longue, leurs pontes étaient plus importantes en taille et en poids et leurs jeunes étaient plus petits que dans le cas des poissons de l'étang piscicole, lesquels cessaient de se reproduire d'octobre à mars, ce qui est caractéristique des populations naturelles de cette région. Les facteurs environnementaux probablement responsables de ces différences sont, notamment : le caractère imprévisible de l'abondance des ressources alimentaires, la mortalité accrue due à la chaleur et une prédation plus importante par les poissons et les oiseaux dans les eaux chauffées. La question de savoir dans quelle mesure ces modifications du cycle vital sont attribuables à des changements génétiques adaptatifs ou plutôt à une plasticité phénotypique reste à examiner.

Received January 24, 1990
Accepted July 23, 1990
(JA446)

Reçu le 24 janvier 1990
Accepté le 23 juillet 1990

Differences in life history characteristics among populations of a species provide material for assessing patterns and mechanisms of microevolutionary change. Life history characteristics may diverge among populations through genetic change in response to some biotic or abiotic aspect(s) of the environment (selection), or through phenotypically plastic responses to local conditions. Divergence may also be influenced by a founder effect or population bottleneck that reduces available genetic variation.

Novel or extreme environments, whether natural or created by man, provide excellent opportunities to evaluate population divergence in life history evolution. Chronic exposure of organisms to anomalous circumstances can provide (1) a perspective of the extreme conditions to which they can adapt, (2) an opportunity to test ideas relative to life history theory or basic natural history of the species of interest, and (3) information to explain the success of "weedy" species in severely disturbed habitats.

In this paper I report on life history differences between populations of the eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae) from an ambient and a thermally elevated habitat in South Carolina. Monthly samples taken over a 2-yr period allow temporal analyses of life history divergence induced by temperature increase and its correlated changes. Populational life history differences are then interpreted with respect to likely selection pressures in these habitats, although there are presently no substantive data to directly test this issue. Whether divergence has a genetic or plastic basis awaits further study.

Methods

Study Organism

The livebearing eastern (*Gambusia holbrooki*) and western (*Gambusia affinis*) mosquitofishes (see Wooten et al. 1988 and Smith et al. 1989 for taxonomic clarification of "mosquitofishes") occur naturally throughout coastal drainages from New Jersey through the Gulf of Mexico to Veracruz, and up the Mississippi Valley to Indiana. They are also widespread throughout the world through active introduction by man (Courtenay and Meffe 1989). They occur in most lentic environments including lakes, ponds, and springs, as well as stream and river margins, and fresh and brackish water marshes. Mosquitofishes have internal fertilization and embryonic development within the female, which has made them excellent subjects for descriptive and hypothesis-testing life history studies (Ferens and Murphy 1974; Bennett and Goodyear 1978; Stearns and Sage 1980; Reznick 1981; Stearns 1983a, 1983b; Vondracek et al. 1988).

The thermal requirements of *G. holbrooki*/*G. affinis* are well known. Mosquitofishes are tolerant of broad temperature ranges, and occur from ice-covered northern lakes (Krumholz 1948) to hot springs (Otto 1973; Winkler 1975). Their growth, reproduction, and general behavior is, of course, related to temperature, with narrower thermal limits for these functions than for survival (Ferens and Murphy 1974; Falke and Smith 1974; Bennett and Goodyear 1978; Shakuntala and Reddy 1979; Wurtsbaugh and Cech 1983; Vondracek et al. 1988).

Study Sites

Studies were conducted on the Savannah River Site, a U.S. federal nuclear production facility on the upper coastal plain near Aiken, South Carolina. Mosquitofish were collected monthly for 2 yr from two contrasting habitats. Risher Pond (=ambient system) is a small (1.1 ha), abandoned farm pond, approximately 50 yr old. There has never been artificial thermal imput to Risher Pond and it is subject to daily and seasonally ambient thermal conditions. Vascular plant growth is lush, invertebrates abound, and the system suitably represents an ambient, productive, southeastern pond.

In contrast, Pond C (=thermal system), constructed over 30 yr ago and approximately 15 km from Risher Pond, is part of the Par Pond cooling reservoir system. Pond C is a 67-ha precooling reservoir that has received thermal effluent (50–70°C) from a nuclear reactor since 1958. The reactor operates through most of the year, with intermittent and unpredictable shutdowns for periods ranging from several hours, to weeks, or occasionally months.

Water temperature in most of Pond C is lethal to fish (>40°C) during reactor discharge, but refugia exist at tributary streams and springs (Block et al. 1984). These refugia are warmer than ambient, but remain below upper lethal limits for fishes, which survive here under even the hottest conditions experienced by the remainder of Pond C; eastern mosquitofish are abundant in these areas. Thermal fluctuations in the Pond C refugia are accompanied by (1) changes in mosquitofish food resources: zooplankton are virtually eliminated during thermal periods (Taylor and Mahoney 1988) and other invertebrates are likewise reduced or eliminated (pers. obs.); (2) intense crowding: mosquitofish, bluegills (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), and several rarer species occur in high densities in these refugia (Block et al. 1984); and (3) heavy predation on mosquitofish: no submergent vascular plants survive in the system and essentially no other structural cover exists for mosquitofish; bass and bluegills can regularly be seen chasing mosquitofish through the shallows, and large numbers of herons and kingfishers prey on them along the shorelines.

Pond C thus provides a unique opportunity to examine life history responses to unpredictable thermal conditions. The study was conducted after 28 yr of thermal effluent; at two to three generations per year (Stearns 1983a), this represents 56–84 mosquitofish generations that experienced selection by hot water and its covariates.

Temperature Data

Temperatures during the study period were not taken because single-point, monthly temperatures would indicate little about the dynamics of Pond C, where diel temperature fluctuations can be sudden and large during reactor operations. Additionally, the immediate temperature at which a fish is collected has little bearing on its measurable life history characters, which are an integrated response to conditions throughout the individual's life, and particularly for the several weeks immediately beforehand. Because continuously recording thermographs were not available during the study, I relied upon previously collected temperature data and detailed knowledge of the reactor operating schedule to determine relative temperatures experienced by the two populations.

Continuous temperature data from earlier dates at the study sites were obtained to illustrate typical daily and seasonal temperatures experienced by fishes (Fig. 1). When the reactor is operating, temperatures in open waters of the Pond C study area typically reach 40–45°C. By then obtaining records for reactor schedules during the study period, I knew precisely when Pond C fish experienced these thermal conditions (top of Fig. 2). When the reactor was operating, water became extremely hot and near lethal within several days; when off, water returned to ambient over a similar period.

Field Sampling

Mosquitofish were sampled by seine and dip net from the same locations on Risher Pond and a refuge area of Pond C, on or near the first of every month from April 1986 through April 1988 ($n = 25$ samples from each system). Mature female mosquitofish (minimum of 20) were haphazardly sampled without regard to reproductive state, but with an attempt to cover the full size range of individuals available. Fish were immediately preserved in 10% Formalin.

Laboratory Procedures

After a minimum of 1 wk in Formalin, specimens were washed in water and stored in 70% ethanol. After standard length (SL) measurement to the nearest 0.1 mm, fish were dissected and the ovary and digestive tract removed. Clutch size (CLUTCH) was determined by counting all embryos and/or mature ova (stages 2–6 of Meffe 1986). Ova that had not yolked, or were partially yolked (stage 1 of Meffe 1985), were considered to constitute the next clutch, and were not included in clutch size. The ovary and soma were then separately dried to constant weight (24–28 h at 80°C) and weighed to the nearest 0.1 mg (reproductive dry weight (RDW) and somatic dry weight (SDW), respectively). Reproductive allocation (RA) was calculated as the percent of total weight devoted to reproduction $\{RA = [RDW/(RDW + SDW)] \times 100\}$.

Analytical Procedures

All statistical analyses were conducted on the Systat® statistical system for personal computers (Wilkinson 1988). Standard procedures were used to calculate means, variances, correlation coefficients, etc. (Sokal and Rohlf 1981). Because CLUTCH, SDW, RDW, and RA are all potentially a function of female size, it is necessary to remove size (SL) as a covariate before comparing these parameters across months or habitats (analysis of covariance (ANCOVA)). However, a fundamental assumption of ANCOVA, equality of slopes, was repeatedly violated. Thus, I chose an alternative approach that standardizes female size and enables direct comparison of life history parameters across months and sites. I calculated the predicted value (and a 95% confidence interval; Sokal and Rohlf 1981) for each parameter at 30 mm SL, using simple linear regressions of ln-transformed data (arcsin square root transformation for percentages) for each parameter in each month. A 30-mm length was chosen because it is close to the grand mean size for both populations (Table 1). Results of these analyses for monthly values of SDW, RDW, RA, and CLUTCH are presented graphically. Each data point is thus the predicted value (with 95% confidence interval) for a 30-mm fish in that habitat for that month.

Results

Thermal Characteristics

Seasonally ambient conditions (typical of Fig. 1A) occurred in Risher Pond whereas Pond C experienced numerous thermal cycles from reactor operations (Fig. 2). Other than specific timing of events, thermal patterns of Pond C are well illustrated by those in previous years (Fig. 1B). Note that Pond C was warm virtually all winter (Fig. 2), with major reactor "down" periods (>2 wk) occurring only during warmer months (June to August in 1986 and April to June in 1987).

Life History Patterns

Because mosquitofish in Risher Pond experienced regionally ambient conditions during the study, they represent a yardstick against which life history patterns of Pond C mosquitofish should be compared. Mean values taken over all samples for the six parameters in each habitat (Table 1a) indicate that a mosquitofish randomly chosen from Pond C was slightly smaller, had a larger clutch, was lighter bodied, and had a greater absolute and proportional reproductive investment than a Risher Pond fish. Mean predicted values for 30-mm fish (Table 1b) approximate the same pattern. These values, of course, only demonstrate general population tendencies, and disregard seasonal changes.

Seasonal patterns for a 30-mm fish were apparent. In Risher Pond, SDW was highest in autumn through early winter and fell to a minimum in April through June, during peak reproduction (Fig. 2A). Pond C fish followed roughly the same pattern, but with less distinct seasonal peaks and troughs, and greater month-to-month variation. The latter was quantified by measuring the absolute difference in SDW between adjacent months for each population. Mean monthly changes in SDW (standard deviations in parentheses) were 5.65 (4.61) for Risher Pond and 9.17 (5.52) in Pond C; these are significantly different values ($t = 2.40$, $P < 0.05$). Thus, mosquitofish in an ambient system experienced smaller monthly fluctuations in somatic weight than did mosquitofish in the thermal habitat.

Risher Pond fish also exhibited predictable seasonality in RDW (Fig. 2B). Peak reproductive weight occurred in May and declined to near zero from October through March, whereupon it rapidly increased again in April. Pond C fish followed the same annual pattern but nearly always had higher values in a given month. This was especially true in winter, when reproduction continued in the thermal system, but at a lower level

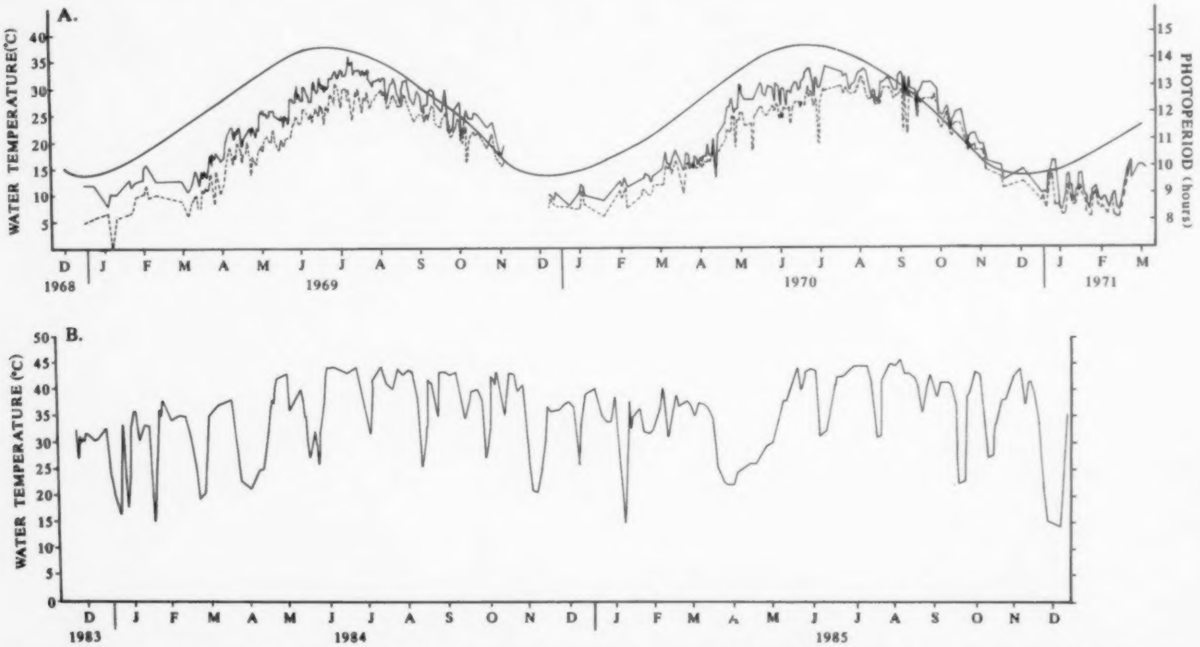

FIG. 1. Thermal histories of Risher Pond and Pond C. (A) Surface water temperatures from Risher Pond showing maximum (solid line) and minimum (broken line) daily temperatures over more than 2 yr. Note the predictable seasonal patterns and relatively minor daily to weekly fluctuations. Also shown is the annual photoperiod at Clemson, SC, approximately 1.5° latitude north of the study areas (from Kish 1974). (B) Mean surface water temperatures from Pond C, near the collection site, in a time span immediately before this study. Note the unpredictable nature of thermal changes in this system due to variation in thermal effluents from reactor operations. The actual collection area, adjacent to where a small feeder stream enters the pond, is typically several degrees cooler.

TABLE 1. Mean values for life history parameters combined for all 25 monthly samples. (a) Mean values (with one standard deviation in parentheses) for all fish, regardless of size. (b) Mean values for 30-mm fish, estimated from separate regressions for each month (calculated as the mean of the predicted value for each individual month).

	SL (mm)	CLUTCH	SDW (mg)	RDW (mg)	RA (%)
(a) Risher Pond	29.98	6.4	106.8	10.9	7.7
(n = 497)	(5.38)	(10.4)	(62.6)	(17.1)	(9.4)
Pond C	29.11	9.5	97.2	17.0	14.3
(n = 500)	(4.84)	(8.2)	(55.9)	(14.9)	(9.6)
t	2.67	5.28	2.53	6.02	12.47
P	0.008	<0.001	0.012	<0.001	<0.001
(b) Risher Pond	—	6.0	95.7	8.5	7.8
Pond C	—	10.7	95.5	17.4	15.0

than in spring or summer. These same patterns are seen in the proportion of biomass invested into reproductive tissue (Fig. 2C).

Largest clutches in Risher Pond (Fig. 2D) occurred in May or June and declined to zero in October through March. Clutch sizes in Pond C peaked over a much wider time frame, from as early as March to as late as October. Individuals in this thermal population also continued to produce offspring through winter, albeit at a lower rate than in spring or summer. They generally produced larger broods than Risher Pond fish except during peak reproduction in the ambient system.

The immediate thermal environment may explain large changes in monthly clutch size in the thermal area. In July 1986, predicted clutch size for a 30-mm fish in Pond C rose sharply, from <13 to 22; this coincided with reactor shutdown and return to ambient temperatures (Fig. 2D). In contrast, a large decline in clutch size (from 17 to 10) occurred in July 1987, coincident with reactor restart after more than 2 mo of ambient conditions. Clutch size then steadily rebounded over the next 3 mo while the reactor was on.

On the population level, the percentage of females reproductively active (carrying a clutch of mature ova or developing embryos) changed seasonally in both habitats (Fig. 3). However, Risher Pond fish routinely dropped to 0% activity in winter whereas Pond C fish were usually above 50%, another indication of reproduction throughout the year in the thermal system. In Fig. 3, percentage reproductive activity is probably underestimated (except for the zero values during winter) because some samples included small fish (20–22 mm) that, although sexually mature, had not yet developed stage 2 through 6 embryos.

Correlations among Life History Parameters

Correlations between SDW and reproductive parameters (Table 2) were all negative, and four of six were significant. This implies a trade-off between somatic and reproductive compartments, indicating that energy or biomass may have been stored in somatic tissue (or as lipid storage) and shunted to the ovary during reproduction. Such a pattern of energy allocation among body compartments was documented for mosquitofish by Reznick and Braun (1987), who demonstrated that lipid storage was important to winter survival and spring reproduction.

Discussion

The life history pattern of Risher Pond mosquitofish is probably characteristic for the region because that system is a typical southeastern pond subject to natural fluctuations in biotic and abiotic parameters. These fish overwintered in a reproductively quiescent state, and increased body weight from late summer through early to midwinter. Somatic weight declined by late winter and early spring and reached a low point during peak reproduction in May. This may imply that food resources were low or depleted in winter, or that low winter temperatures reduced metabolic rates to a point that digestion was inefficient or impeded (Wurtsbaugh and Cech 1983). Whatever the reason, mosquitofish from such an ambient habitat entered their peak reproductive period in relatively poor somatic condition and put a great deal of available energy, presumably including stored energy (Reznick and Braun 1987), into reproduction. Their condition recovered during summer, when resources were probably at their maximum, and higher temperatures resulted in normal metabolic activity.

There were strong and consistent deviations from this pattern in mosquitofish from Pond C, presumably a result of thermal supplements and their covariates such as resource type and availability. The most obvious life history change was continued reproduction throughout winter in the thermal pond. More subtle differences included higher overall reproductive activity in nearly all months, less pronounced seasonal changes, and greater monthly fluctuation in somatic condition.

Apparent trade-offs between life history characters (Table 2) were strong in Risher Pond but weak in Pond C mosquitofish. Trade-offs may have been more possible in the predictable ambient system, where annual energy inputs are certainly more tractable. Energy storage (as lipids) in autumn for spring reproduction appears to be important in mosquitofish (Reznick and Braun 1987; G. K. Meffe and F. F. Snelson Jr., unpubl. data). Such storage can be relied upon in an ambient system, where food and temperature are seasonally predictable. In the unpredictable thermal system, in which food resources may be abundant at one point and then suddenly annihilated (Taylor and Mahoney 1988), individuals may have acted in a more opportunistic fashion and relied on external sources of energy when available, rather than seasonally shunting energy among body compartments.

There were also significant size differences (both length and dry weight) of offspring from the two habitats; Pond C neonates are generally smaller than Risher Pond neonates (Meffe 1990). This thermally affected population thus produced more and smaller offspring, and invested a greater proportion of biomass (and presumably energy) into reproduction than did fish in an

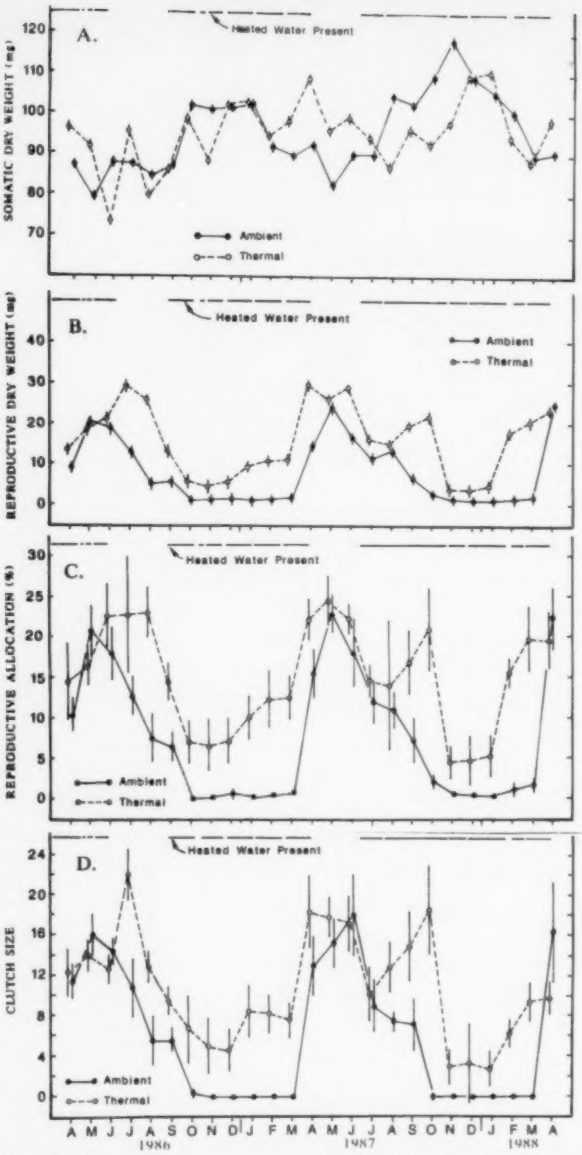

FIG. 2. (A) Somatic dry weight, (B) reproductive dry weight, (C) reproductive allocation, and (D) predicted clutch size for a 30-mm female over the study period. Note that the reactor operation record is included at the top of each figure.

ambient system. Conversely, thermally affected fish had smaller somatic investments.

These patterns make sense in terms of a life history "strategy." In a predictable and benign system such as Risher Pond, especially with its apparently low predation, individuals have a high likelihood of surviving to the next reproductive period, and may invest smaller proportions of the energy budget into each reproductive episode, thereby reducing the associated "cost of reproduction" in terms of higher mortality rates (Williams 1966). In an unpredictable and harsh system such as Pond C, it makes little sense to reduce current reproductive investment for the benefit of future reproduction, as that latter opportunity has a comparatively low chance of occurring at all. Conditions in Pond C may select for maximal reproductive effort in fishes whenever the opportunity presents itself, as the individual has a low likelihood of surviving to the next opportunity. This can be partially tested by comparing age structures and longevities in the two systems; I predict much shorter life spans (few old fish) in Pond C.

An obvious problem in a study such as this is lack of replication of systems. Although major life history differences exist between these populations, they cannot be unequivocally attributed to thermal differences without first replicating sites. However, thermal sites do not abound, and this was the only thermal system available at this time within the local geographic range; sampling of more than one population from each site would only have been pseudoreplication (Hurlbert 1984) and not helpful at all to this problem. However, previous studies that included other thermal systems in the region (when those systems were still available) demonstrate similar life history changes associated with the thermal condition (Ferens and Murphy 1974; Bennett and Goodyear 1978). Also, a parallel study on bluegills of Pond C and an ambient system indicates similar life history alterations (R. U. Fischer, Savannah River Ecology Laboratory, pers. comm.). Thus, although I cannot say with certainty that the population differences documented herein are due to thermal differences and their covariates (food, cover, etc.), that seems the most parsimonious explanation. Consequently, the following scenario is my speculative interpretation of selection pressures resulting in the life history changes outlined. These are presented only as testable predictions.

There are probably two major selective challenges for Pond C fish. First, food resources are less stable and predictable in the thermal system. Pond C zooplankton undergo cycles of extinction and recolonization during hot and ambient periods, respectively (Taylor and Mahoney 1988), and the timing of these periods is unpredictable. Other potential food items, including algae and macroinvertebrates, follow the same pattern (pers. obs.). Second, mosquitofish in Pond C experience higher mortality rates from hot water and probably also from predation by fish and wading birds. Thermally induced mortality is high in Pond C, especially during reactor restart after a prolonged ambient period; thousands of mosquitofish, especially large, pregnant females, are killed by these high temperatures (J. M. Aho, Savannah River Ecology Laboratory, pers. comm.). Lack of vascular plants or other cover in Pond C must contribute greatly to susceptibility of mosquitofish to both aquatic and aerial predation, as does their concentration into small refugia with predators during thermal periods (Block et al. 1984).

Adult mortality is probably higher than newborn or juvenile mortality in Pond C because adults tend to venture into deeper (and thus hotter) water whereas newborns usually congregate in the shallowest and coolest pockets which are more protected from high temperatures and fish predation. Also, predation from bass and birds is likely to be higher on adults, which are more readily visible, occur in deeper waters more accessible to bass, and offer a higher energetic return to large predators. Herons selectively prey on large female mosquitofish (Britton and Moser 1982).

In contrast, Risher Pond fish are not subject to these thermally related phenomena. Lethal temperatures do not occur in Risher Pond, even on the hottest summer days. Predation from fish and birds is probably far less intense here as well. Largemouth bass are rare in the system; during a drawdown in 1983,

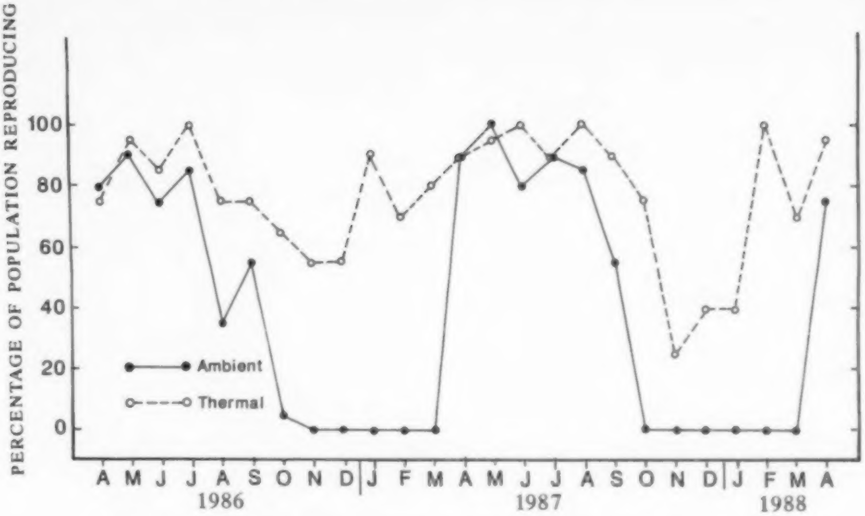

FIG. 3. Percentage of the population pregnant each month for both populations.

TABLE 2. Pearson's product–moment correlation coefficients for life history parameters, by habitat of origin. *$P < 0.05$; **$P < 0.01$.

	SDW	RDW	CLUTCH
	(a) Risher Pond		
RDW	−0.603**		
CLUTCH	−0.645**	0.976**	
RA	−0.644**	0.996**	0.978**
	(b) Pond C		
RDW	−0.194		
CLUTCH	−0.226	0.887**	
RA	−0.428*	0.964**	0.863**

three adult bass were recorded, with no indication of recruitment (J. W. Gibbons, Savannah River Ecology Laboratory, pers. comm.). I have recorded one warmouth (*Lepomis gulosus*) in the pond, and occasionally see a centrarchid, which could indicate some level of fish predation; nevertheless, centrarchid population densities in Risher Pond must be an order of magnitude lower than those in Pond C. Piscivorous birds are not abundant at Risher Pond (probably because it is too deep for wading foragers), and extremely heavy submerged and emergent vegetation along the entire shoreline and over much of the surface provides good cover for mosquitofish from potential aerial and aquatic predators. However, I have observed banded water snake (*Nerodia fasciata*) predation on an adult female *G. holbrooki* in Risher Pond.

Divergence of Pond C mosquitofish life history patterns therefore appears to reflect the biotic and abiotic constraints presented by that unique system. Because mortality rates are probably higher in the thermal system, especially among reproducing adults who are prey for bass and birds, mosquitofish put more energy into reproduction and less into somatic growth and maintenance. More, but smaller, offspring are produced, with reproduction a year-round event. Predictions of life history patterns in a system with higher adult than juvenile mortality include early maturity, large clutches of small offspring, and high reproductive investment (Law 1979; Michod 1979); the latter patterns are documented herein, and I predict that Pond C mosquitofish mature at an earlier age than do fish from ambient habitats, and that old fish are rare in Pond C. Both predictions will be tested.

It is interesting that reproduction ceased by October 1 in the ambient habitat. Water temperature at that time was higher than in April (Fig. 1A), when mosquitofish reproduced, and equivalent to May temperatures, when reproduction was strongest. Temperature alone therefore did not cause reproductive cessation in autumn. Decreasing photoperiod (Fig. 1A) probably played a role, as photoperiod on October 1 was equivalent to that on March 7, when fish probably just start to develop their eggs after winter quiescence. It makes sense that mosquitofish should stop reproducing by October 1 because progeny born at this time would have to face cold temperatures, shortening daylengths, and presumably decreasing food supplies early in life. Any reproductive episode so late in the year may have a high likelihood of failure, and it is probably more beneficial to put that energy into storage compartments (lipids or soma) for winter survival and spring reproduction, as documented by Reznick and Braun (1987).

Artificially increasing temperature during winter in Pond C, while photoperiod remains natural, provides an opportunity to examine the relative importance of these parameters in annual reproductive cycles. Although photoperiod may play a role in stopping reproduction in autumn (Fig. 1A), higher water temperatures can apparently override photoperiod to some extent because mosquitofish continued to reproduce during winter in Pond C. However, reproduction was still affected to some degree by photoperiod because clutch size and reproductive masses declined in winter, as per the basic pattern in ambient conditions. Controlled laboratory experiments are needed to separate these effects and to determine whether temperature and photoperiod influences can be further decoupled.

It is presently unknown to what extent the life history alterations presented here are simply plastic responses to the immediate environment, versus genetic change. This should be tested either in a "common garden" laboratory experiment (sensu Clausen et al. 1948) or through reciprocal field transplants designed to separate genetic from phenotypic responses. There are genetic differences between Pond C and Risher Pond mosquitofish, as detected through electrophoretic studies: heterozygosity is about twice as high in Risher Pond as in Pond C, and there are fixed allelic differences as well (Smith et al. 1983). This suggests at least a basis for genetic influences on life history changes.

These patterns are similar to life history differences among guppy (*Poecilia reticulata*) populations in Trinidad (Reznick and Endler 1982), for which a genetic basis was demonstrated (Reznick 1982). In that system, guppies exposed to heavy predation on adults matured earlier and had larger broods, smaller offspring, and higher reproductive allocation than did populations exposed to low adult or juvenile predation. This pattern was retained in laboratory-reared F_2 individuals. Stearns (1983a) demonstrated interpopulational differences in western mosquitofish introduced early this century into a series of Hawaiian ponds. Fish from fluctuating reservoirs had larger clutches and higher reproductive allocation and were heavier and larger than fish from stable reservoirs, although a great deal of variation was not explainable by the fluctuating/stable dichotomy. There was a genetic basis for some life history differences (Stearns 1983b), indicating that they can arise rapidly (within this century). Thus, it is reasonable to suspect that life history divergences documented herein may have a genetic basis, but this remains to be tested directly.

Acknowledgments

I thank Gwen Keller, David Kling, Jim Knight, and especially Amy Powell for assistance with field collections and dissections. The manuscript was improved through the comments of John Aho, Justin Congdon, Robert Fischer, David Reznick, and several referees. John Aho and Judy Greene provided water temperature data for Pond C and Risher Pond, respectively. Research was supported by contract DE-AC09-76SROO-819 between the U.S. Department of Energy and the University of Georgia.

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Acoustic Analysis of Diel Vertical Migration Behavior of *Mysis relicta* and Kokanee (*Oncorhynchus nerka*) within Okanagan Lake, British Columbia

David A. Levy

Levy Research Services Ltd., 102-2221 Folkstone Way, West Vancouver, B.C. V7S 2Y6, Canada

Levy, D. A. 1991. Acoustic analysis of diel vertical migration behavior of *Mysis relicta* and kokanee (*Oncorhynchus nerka*) within Okanagan Lake, British Columbia. Can. J. Fish. Aquat. Sci. 48: 67–72.

Dual-beam acoustic surveys of Okanagan Lake suggested active diel vertical migrations of *Mysis relicta* and kokanee (*Oncorhynchus nerka*) within the pelagic zone. *Mysis relicta* were situated between 90–150 m during the day and migrated upwards into the thermocline region of the water column at night. Two groups of kokanee targets were detected. The first undertook a diel vertical migration and coalesced at dusk with a second, shallow-oriented group of targets. Daytime target strength estimates taken while the two groups were vertically segregated in the water column suggested an 8–12 db lower target strength of the deeper group. The results provide acoustic evidence for a smaller body size in the deeper group and the occurrence of an ontogenetic shift in diel migratory behavior of kokanee within Okanagan Lake. Diel comparisons of depth distribution suggested spatial segregation of *Mysis* and kokanee over much of the diel cycle.

Des études acoustiques à double faisceau réalisées au lac Okanagan donnent à penser qu'il existe des migrations verticales nycthémérales actives de *Mysis relicta* et du saumon kokanee (*Oncorhynchus nerka*) dans la zone pélagique. *Mysis relicta* a été localisée à 90–150 m durant le jour et on a constaté une migration ascendante au cours de laquelle l'organisme atteignait la thermocline de la colonne d'eau pendant la nuit. Deux groupes de cibles de saumons kokanee ont été détectés. Le premier groupe a commencé une migration verticale nycthémérale et, au crépuscule, s'est mêlé avec un deuxième groupe de cibles, qui vit plutôt dans les couches superficielles. Des estimations de l'effectif diurne des cibles effectuées pendant que les deux groupes étaient séparés verticalement dans la colonne d'eau indiquent que l'effectif des cibles est inférieur (8–12 db) pour ce qui est du groupe vivant en plus grande profondeur. Les résultats obtenus par étude acoustique montrent que la taille corporelle est inférieure dans le groupe vivant en plus grande profondeur et indiquent la survenue d'une modification ontogénétique dans le comportement migratoire nycthéméral du salmon kokanee dans le lac Okanagan. Les comparaisons nycthémérales de la distribution des profondeurs laissent croire qu'il existe une ségrégation spatiale de *Mysis* et du saumon kokanee pendant une grande partie du cycle nycthéméral.

Received December 8, 1989
Accepted July 23, 1990
(JA393)

Reçu le 8 décembre 1989
Accepté le 23 juillet 1990

Juvenile sockeye salmon (*Oncorhynchus nerka*) frequently undertake diel vertical migrations in their freshwater nursery lakes (Narver 1970; Levy 1990). The usual pattern is for the fish to spend daytime periods deep in the water column and to ascend into shallow surface waters during crepuscular and nocturnal portions of the diel cycle. In contrast, kokanee (the lake-locked form of sockeye salmon) undertake reversed diel vertical migrations in at least several lakes, including Nicola (Northcote et al. 1964; Northcote 1967), Babine (McDonald 1969), and Pend Oreille (Rieman and Bowler 1980). These subadult (1–4 yr old) fish occupy shallow water during the day and descend at night. There is no information available on juvenile (0–1 yr old) kokanee diel vertical migrations within such systems, nor do we know whether the juvenile fish undertake reversed diel vertical migrations, or behave like juvenile sockeye, shifting their diel vertical migratory behavior later in life.

Mysis relicta also undertake diel vertical migrations in limnetic environments ascending at dusk, and descending at dawn, through vertical distances of tens of meters (Beeton 1960; Beeton and Bowers 1982). *Mysis* are heavily consumed by larger size-classes of kokanee (Northcote 1973; Lasenby et al. 1986) and may thus influence the diel vertical distribution of kokanee within the pelagic water column. Another planktivore, the alewife (*Alosa pseudoharengus*), undertakes diel vertical migrations in Lake Michigan which parallel those of *Mysis* within the water column (Janssen and Brandt 1980).

Mobile pelagic organisms are notoriously difficult to sample quantitatively. For example, density estimates of *M. relicta* in lakes can vary by fivefold (Nero and Davies 1982) or an order of magnitude (Grossnickle and Morgan 1979) depending upon the choice of sampling method. Pelagic fish sampling efficiency can also be influenced by environmental conditions. Thus, nocturnal trawling efficiency for juvenile sockeye salmon in Great Central Lake, under clear sky and full moon, was only 6.4% of that during overcast sky and dark of moon (Robinson and Barraclough 1978).

An alternative to net sampling methods for quantitative studies within the pelagic zone is offered by acoustic measurement systems, provided that certain critical conditions are satisfied (Thorne 1983). Recently, dual-beam hydroacoustic procedures have been effectively applied for quantitative surveys of diverse pelagic organisms, including juvenile sockeye in a freshwater nursery lake (Burczynski and Johnson 1986), marine squid (Jefferts et al. 1987), and marine euphausiids (Greene et al.

1988, 1989). A major advantage of the dual-beam system is the capability for in situ target strength estimates (Ehrenberg 1983). Target strengths can be related statistically to fish size (Love 1971), thereby making it possible to infer the size distribution of echo targets, provided that these are not confounded by attitude (Buerkle 1987) or other effects. Since kokanee occur in the pelagic zone in discrete annual age cohorts (Parkinson 1988), it may be feasible to derive ecologically relevant kokanee size information utilizing dual-beam acoustic measurements.

The purpose of the present study was to simultaneously monitor the diel vertical migrations of *Mysis* and kokanee in the pelagic zone of Okanagan Lake, British Columbia, to determine (1) the degree of distributional overlap of *Mysis* and kokanee over the diel cycle, (2) whether there is an ontogenetic shift in kokanee diel vertical migration behavior, and (3) whether it is feasible to acoustically separate cohorts of kokanee by dual-beam analyses.

Study Area and Methods

The site chosen for study was a deep (>200 m depth) mid-lake site adjacent to Okanagan Centre on Okanagan Lake (Fig. 1). Okanagan Lake is a large (100 km length by 3 km width approx.) oligotrophic lake situated in the Okanagan Valley region of British Columbia. Limnological and biological conditions with the lake have been characterized by Clemens et al. (1939) and Stockner and Northcote (1974). Pelagic mid-water trawl surveys are conducted annually within Okanagan Lake by the B.C. Ministry of Environment (E. Parkinson, B.C. Ministry of Environment, Recreational Fisheries Program, 2204 Main Mall, University of British Columbia, Vancouver, B.C. V6T 1W5, pers. comm.). About 99% of the fish captured in trawl nets deployed in open water are kokanee (Parkinson 1988), and the lake supports an important recreational kokanee fishery. Of the 14 different fish species which occur within Okanagan Lake (Clemens et al. 1939), kokanee are numerically predominant within the pelagic zone and are probably at least 3 orders of magnitude more numerous than all other pelagic fish species combined (B. Shepherd, B.C. Ministry of Environment, 3547 Skaha Lake Road, Penticton, B.C. V2A 7K2, pers. comm.)

The hydroacoustic system adopted for data collection was a BioSonics model 105 420-kHz dual-beam echosounder together with a 6°/15° dual beam transducer. Acoustic signals (0.4-ms pulse width generated at a rate of $2 \cdot s^{-1}$) were amplified at 40 log R time-varied gain, digitized with a pulse code modulator (PCM) device and then taped on beta-format video cassette recorder (VCR) tape in the field for subsequent laboratory analysis and data processing.

Echo signals were monitored in the field on an oscilloscope and chart recorder. By monitoring the detected output from an amplifier playback interface, it was possible to ensure that echo signals were effectively recorded on VCR tape and that the receiver gain was adjusted so as to achieve echo voltages from fish targets between 0.5 and 3 V (V_{rms}), thus maximizing signal to noise ratios while simultaneously avoiding tape saturation levels (10 V). Nunnallee (1973) described the trade-off between system gain and tape saturation. At regular intervals during data collection, the voltage of a continuous wave calibration tone was measured and recorded. This procedure permitted standardized playback gain adjustment prior to echo signal processing.

The echosounder system was housed under the canvas cover of a 5-m fiberglass outboard boat. The transducer was deployed from a rotatable pipe mount, fixed amidships. Transects of 10 min duration, at a velocity of $2\ m \cdot s^{-1}$ in an irregularly directed fashion, were undertaken to characterize the echo target depth distribution at a given point during the diel cycle. The transects were strategically distributed so as to detect changes in vertical depth position of migrating organisms. Most intensive sampling was undertaken at dusk and dawn when the animals were actively migrating vertically. A total of 20–25

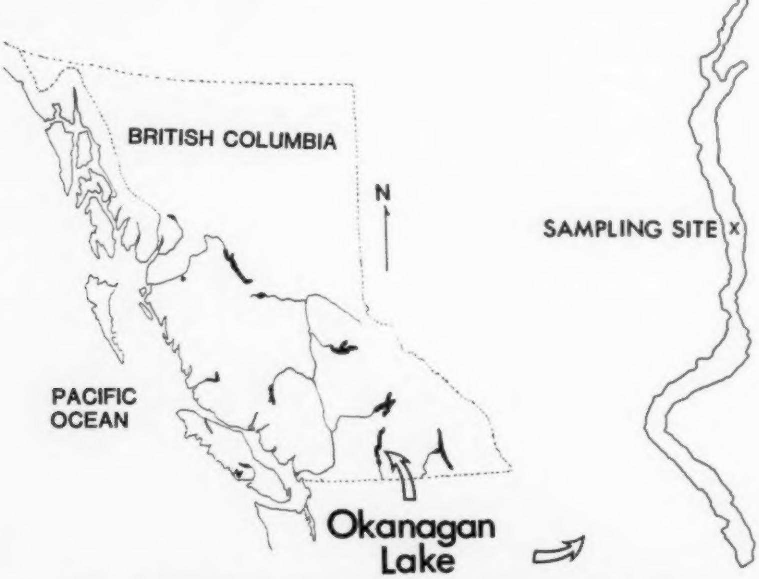


FIG. 1. Echo sounding location within Okanagan Lake, British Columbia.

10-min transects were undertaken to characterize diel cycles in vertical migration during summer and fall periods of the year.

Processing of dual beam echo data was undertaken with a BioSonics model 181 Dual Beam Processor, using recommended pulse amplitude and duration criteria (BioSonics Inc. 1985) to filter out multiple (schooled) fish echo targets. Data were transferred to computer disk and fish target strengths were estimated by the BioSonics "TS" program. Voltage thresholds during analysis were set at 100 mV above the echo returns produced by mysids (measured on an oscilloscope) so as to restrict target strength analyses to fish echo targets.

Results

Echograms collected from Okanagan Lake over the diel cycle showed dramatic differences in appearance (Fig. 2). Back-scattering was observed from objects throughout the upper 150 m of the pelagic water column. There were several distinct groups of echo targets present (Fig. 2A). The predominant scattering layer formed a dispersed group of targets centered at 100–120 m by day and ascended to within 15 m of the surface at night. This was followed by a rapid dawn descent such that depth distribution was significantly lower at the end, than the beginning, of several 10-min transects (e.g. T = 06:30 on Fig. 2A). The back-scattering was observed on the oscilloscope as a broad band of low voltage returns, suggesting the combined echo reflections from a large biomass of small-bodied organisms. These echo returns were ascribed to the presence of *M. relicta* within the water column and suggest an extensive (70–90 m amplitude) diel vertical migration of this freshwater mysid within Okanagan Lake. Echograms produced by vertically migrating *M. relicta* within Lake Michigan (Bowers and Grossnickle 1978) were similar in general appearance to those obtained during the present study.

Mysis relicta were initially introduced into Okanagan Lake in 1966 (Lasenby et al. 1986) and have successfully colonized the lake, although there are no current estimates of their abundance within the system. Comparable mysid diel vertical migration patterns were observed during four summer–fall diel sampling periods during 1987.

The upper limit to the ascent of mysids was evidently controlled by temperature and there was a discrete discontinuity in nocturnal mysid distribution at the thermocline (Fig. 2B). Similar effects of temperature limiting the upper vertical distribution of *M. relicta* in the water column have been observed in Lake Huron and Lake Michigan (Beeton 1960).

While the majority of the mysids within Okanagan Lake evidently undertook diel vertical migrations and concentrated in relatively shallow water at night, nocturnal echo returns from *Mysis* were also detected within deep (100 m depth) water (T = 19:50 on Fig. 2A). These results suggest that some of the mysids within Okanagan Lake only undertook minor vertical movements over the diel cycle.

There were two distinct groups of fish targets present on the echograms. The first was present in shallow (15–25 m) water depths by day, while the second, in deep water during the day, migrated vertically over the diel cycle in a similar fashion as juvenile sockeye salmon in their freshwater nursery lakes (Levy 1990). The latter group evidently ascended from a daytime depth of 50–80 m (T = 14:05 on Fig. 2A) and merged together with the shallow water group at night (T = 19:10 on Fig. 2A). At dawn, the second group descended beneath the shallow water group (T = 07:10 on Fig. 2A) and gradually reassumed the daytime depth level.

Target strengths of individual fish echoes were computed in order to make inferences about the size distribution of the two groups of fish targets. Separate results were computed for day and night transects. During each of the four sampling dates, there was a bimodal depth distribution in the frequency of daytime echo targets (Fig. 3). There was also a difference in the mean target strength of the two spatially separated groups of echo targets, such that the mean of the deeper group of fish echoes was 8–14 db smaller than the mean of the shallow group (Fig. 3). These results suggest that the deeper group of vertically migrating fish targets in Okanagan Lake were smaller in body size, on average, than the shallow group of fish targets.

At night when the two groups of fish targets overlapped spatially, separate modes in target strength distribution could not be resolved (Fig. 3). This may be due to a homogeneous vertical depth distribution of different-sized animals, or alternatively to an inherent inability to resolve target strength modes when the variance is large (variance in target strength is greater than variance in length or weight due to effects of target attitude; see Burczynski and Johnson 1986).

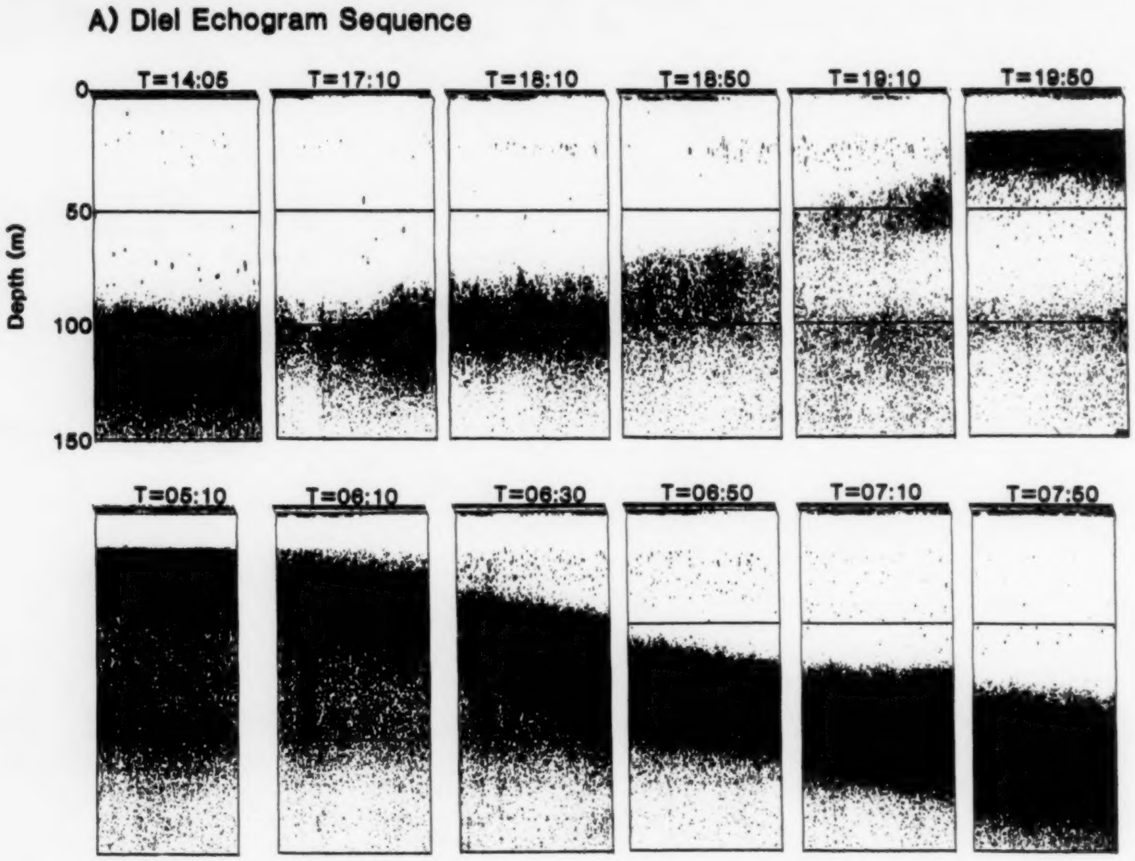
Discussion

Acoustic methods were effective for assessing kokanee and *Mysis* diel vertical migrations in the pelagic zone of Okanagan Lake. *Mysis* undertook rapid, large-amplitude diel vertical migrations (Fig. 2) which would be difficult to describe accurately using traditional plankton sampling methods. Fish net avoidance under illuminated conditions (Robinson and Barraclough 1978) also precludes the application of trawl sampling for description of kokanee diel vertical migrations within Okanagan Lake.

Mysis within Okanagan Lake required 100 min to cover vertical distances of approximately 80 m during their diel vertical migration (Fig. 2). Maximum swimming speeds of *Mysis* within Lake Michigan were estimated at 0.8 $m \cdot min^{-1}$ (Beeton 1960), similar to those observed during the present study. The maximum amplitude of diel vertical migration by *Mysis* within Lake Huron was 96 m (Beeton 1960).

The hydroacoustic results provide qualitative evidence for an ontogenetic shift in vertical migratory behavior of kokanee within Okanagan Lake. Small fish (juveniles) migrate vertically in a similar fashion as juvenile sockeye. Later in life, the kokanee alter their behavior and remain in relatively shallow water during daylight hours. Angling practices within Okanagan Lake partially support this interpretation, since adult and subadult kokanee are vulnerable to shallow surface-deployed hook-and-line fishing gear during daylight hours.

An alternative explanation, that reductions in daytime target strength of deeper fish targets in the water column are due to pressure effects on the swimbladder (Blaxter and Batty 1991), is unlikely because of the magnitude of the observed target strength differences. During the present study, measured target strengths of the deeper group of fish echoes were 8–14 db smaller than the mean of the shallow group (Fig. 3). In another study, Edwards and Armstrong (1983) measured a 2–3 db reduction in herring target strengths in three of four net cage experiments after lowering the fish from 17.5 to 47.5 m. Herring and salmon swimbladders probably respond to pressure in similar ways, since both groups are physostomous. It is there-


A) Diel Echogram Sequence

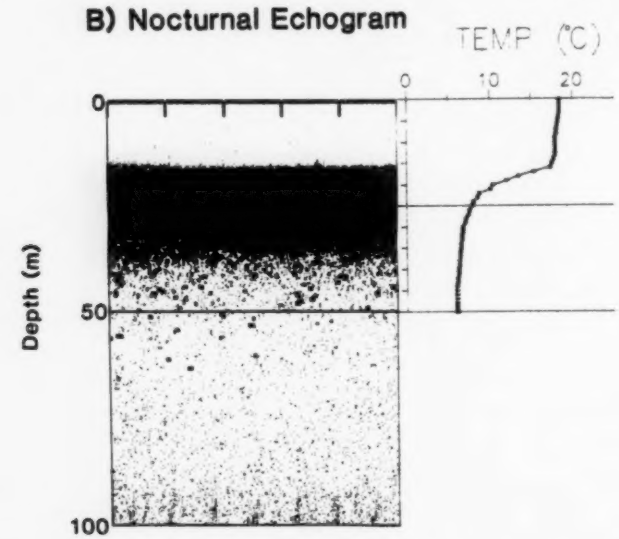

B) Nocturnal Echogram

FIG. 2. (A) Diel echogram sequence from Okanagan Lake on September 30 – October 1, 1987, and (B) nocturnal echogram in relation to the temperature profile. Numbers above echograms indicate time of sampling (PDST).

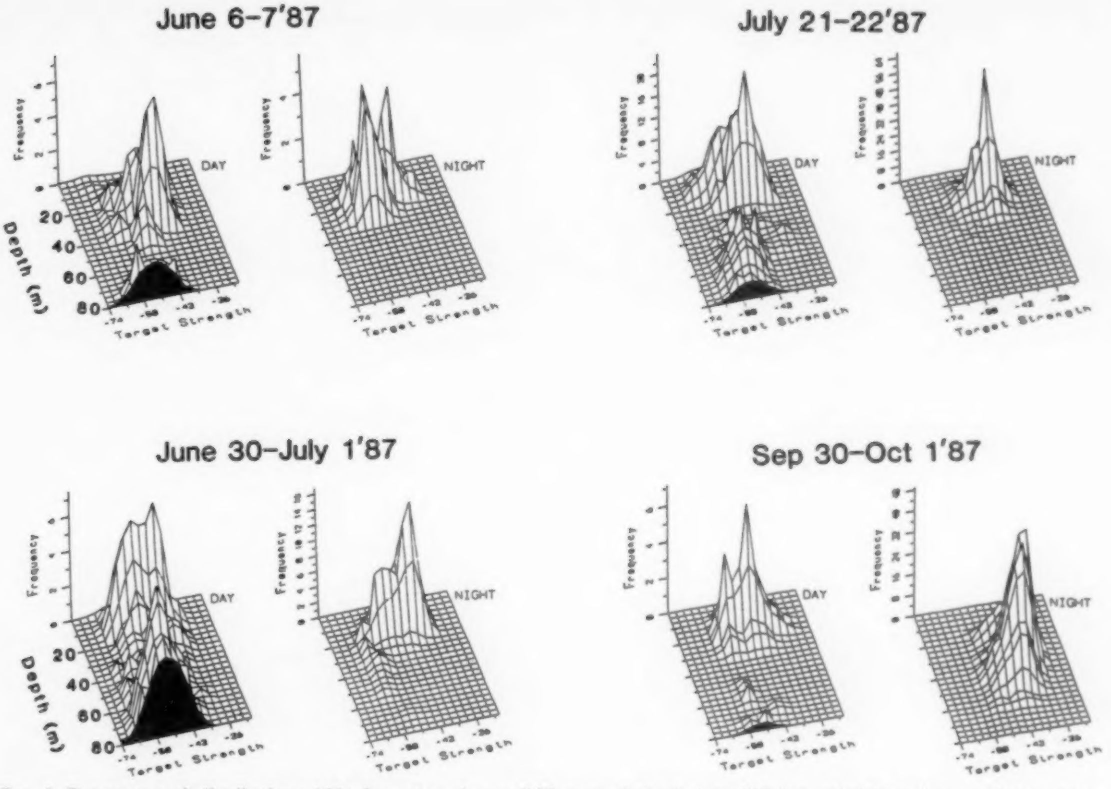


FIG. 3. Target strength distribution within the water column of Okanagan Lake during 1987 day and night hydroacoustic transects.

fore unlikely that the reduced target strength of deeper fish targets in Okanagan Lake is due solely to a pressure effect.

The occurrence of an ontogenetic shift in kokanee diel vertical migratory behavior is consistent with the antipredation window model developed by Clark and Levy (1988). If one assumes an inverse relationship between piscivore-induced mortality risk and body size, and a direct relationship between plankton food requirements and body size, it follows that larger-bodied individuals would spend a greater portion, if not all, of their diel time budget in shallow water. Since planktivorous kokanee are often food limited within freshwater (their growth rate is generally much slower than that of their marine sockeye counterparts), bioenergetic benefits (Brett 1971) could be achieved by large-bodied kokanee by occupying metalimnetic depth strata where temperatures optimize growth, provided that this behavior was not outweighed by the additional predation risk from visual-feeding piscivorous fish within the euphotic conditions of shallow depth strata.

Kokanee within Okanagan Lake evidently feed heavily on *Mysis* once the fish surpass a critical size threshold. Analysis of kokanee stomach contents obtained from anglers on Okanagan Lake between May and June of 1987 and 1988 (C. Bull, B.C. Ministry of Environment, 3547 Skaha Lake Road, Penticton, B.C. V2A 7K2, unpubl. data) showed that small kokanee between 220 and 270 mm fork length (n = 34) fed exclusively on microcrustacea, while those between 340 and 540 mm fork length (n = 49) fed exclusively on *Mysis*. These data suggest that kokanee shift their feeding behavior as they develop, switching from microcrustacea to *Mysis* at a fork length of approximately 300 mm. Similar ontogenetic shifts in *Mysis* exploitation by kokanee over 300 mm also occur in Kootenay Lake where large kokanee (>300 mm) fed heavily upon *Mysis* throughout the entire year (Northcote 1973).

Vertical migration of *Mysis* effectively segregates them from the larger planktivorous kokanee in the water column for much of the diel cycle and may serve as an effective form of pelagic concealment. Kokanee rely primarily on visual foraging cues for prey detection and capture. Diel vertical migration in *Mysis* may thus serve as a predator avoidance mechanism by restricting the amount of time spent under illuminated conditions in shallow water when kokanee searching efficiency would be maximized. Other factors which may play a role in diel vertical migrations of *Mysis* include feeding and temperature effects on metabolic rate (Beeton and Bowers 1982) or physiological adaptation to specific light intensities.

Unlike alewife diel vertical migrations in Lake Michigan, which coincided with those of *Mysis* over the diel cycle (Janssen and Brandt 1980), kokanee in Okanagan Lake remained vertically segregated from *Mysis* for much of the diel cycle. A better understanding of the causes for observed differences in planktivore diel migratory behavior may provide insight into the mechanisms controlling fish production in pelagic environments.

Acknowledgements

I wish to thank Dr. T. G. Northcote who supervized the research at The University of British Columbia, E. Parkinson and C. Bull of the B.C. Ministry of Environment who supplied technical assistance, and R. L. Johnson of Canadian BioSonics Ltd. who provided access to echo signal processing hardware. Research funding was provided by two Department of Fisheries and Oceans subvention awards to Dr. T. G. Northcote, as well as Natural Sciences and Engineering Research Council of Canada postgraduate scholarship.

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Estimating Michaelis–Menten Parameters and Lake Water Phosphate by the Rigler Bioassay: Importance of Fitting Technique, Plankton Size, and Substrate Range

E. Bentzen and W. D. Taylor

Department of Biology, University of Waterloo, Waterloo, Ont. N2L 3G1, Canada

Bentzen, E., and W. D. Taylor. 1991. Estimating Michaelis–Menten parameters and lake water phosphate by the Rigler bioassay: importance of fitting technique, plankton size, and substrate range. Can. J. Fish. Aquat. Sci. 48: 73–83.

Evaluation of the Michaelis–Menten parameters V_m and K_t for phosphate (PO_4^{3-}) uptake by plankton is difficult because of differences in nutrient uptake by the various species. Studies using simulated data have shown that fitting the equation by nonlinear regression is preferable to linearized transformations for estimating V_m and K_t; however, the actual velocity of PO_4^{3-} uptake by lake plankton cannot be measured because the ambient concentration of PO_4^{3-} (P_n) is unknown. For natural plankton, a better fit is demonstrated using another nonlinear model which fits the uptake rate constant for $^{32}PO_4^{3-}$ directly to the PO_4^{3-} added in the kinetic experiment, and the mixed community effect is minimized by isolating different plankton size fractions. Another consequence of not knowing P_n is that the estimate of K_t cannot be distinguished from P_n, and the sum of the two is always obtained. The low substrate region of the Michaelis–Menten curve is used in the Rigler bioassay as a means of estimating the upper bound to P_n. Error in the uptake rate constants influences the estimate of the Rigler P_n, and the Rigler maximum P_n is equal to the estimated ($K_t + P_n$) of the 0.2–1 μm size fraction.

L'évaluation des paramètres de Michaelis–Menten, V_m et K_t, pour l'absorption de phosphate (PO_4^{3-}) par le plancton est difficile à cause des différences interspécifiques relatives à l'absorption des nutriments. Des études utilisant de données simulées ont montré qu'il est préférable d'ajuster l'équation par régression non linéaire plutôt qu'au moyen de transformations linéarisés pour l'estimation de V_m et de K_t; cependant, la vitesse réelle d'absorption du PO_4^{3-} par le plancton dans les lacs ne peut être mesurée parce que la concentration ambiante de PO_4^{3-} (P_n) est inconnue. Pour le plancton en milieu naturel, on obtient de meilleurs résultats en utilisant un autre modèle non linéaire qui fait correspondre la constante du taux d'absorption du $^{32}PO_4^{3-}$ directement avec le PO_4^{3-} ajouté dans l'expérience cinétique; l'isolement de différentes classes de taille permet de réduire l'effet de la diversité du plancton. Le fait de ne pas connaître P_n fait aussi que l'estimation de K_t ne peut être distinguée de P_n: on obtient toujours la somme des deux. La région de la courbe de Michaelis–Menten correspondant à de faibles concentrations de substrats est utilisée dans le cadre du bio-essai de Rigler pour estimer la limite supérieure de P_n. Une erreur dans les constantes du taux d'absorption influe sur l'estimation du P_n de Rigler, et le P_n maximum de Rigler est égal à la valeur estimée de (K_t + P_n) de la fraction dont la taille varie entre 0,2 et 1 μm.

Received February 28, 1990
Accepted July 24, 1900
(JA490)

Reçu le 28 février 1990
Accepté le 24 juillet 1990

The uptake of limiting nutrients by limnetic plankton can be described by the Michaelis–Menten equation for enzyme kinetics:

$$(1)\quad v_i = \frac{V_m \cdot S_i}{(K_t + S_i)}$$

where v_i is the velocity of nutrient uptake at substrate concentration S_i, V_m is the maximum velocity observed when all uptake sites are saturated with substrate, and K_t is a constant equal to the concentration of substrate at which $v_i = V_m/2$. The uptake of phosphate (PO_4^{3-}) by freshwater planktonic algae and bacteria has been shown to conform to this equation when the PO_4^{3-} concentration is low. Individual plankton species possess different values of K_t and V_m, as has been demonstrated in culture studies (Nalewajko and Lean 1980; Cembella et al. 1984; Button 1985). However, even an assemblage of lake plankton generally will follow Michaelis–Menten kinetics; the observed uptake parameters, V_m and K_t, are means for the entire population (Lean and White 1983).

The Michaelis–Menten parameters have been used as indicators of the demand for phosphate and to compare the relative abilities of plankton to sequester phosphate under conditions of limiting PO_4^{3-} (Cembella et al. 1984). A low value of K_t is indicative of a high affinity of the plankton for phosphate, and the abilities of different plankton to compete for PO_4^{3-} have been assessed by comparison of either K_t or the ratio of K_t to V_m (Healey 1980; Smith and Kalff 1982). Lean (1984) developed a phosphorus deficiency index from the ratio of V_m to the maximum rate of photosynthesis.

No reliable chemical method exists for the estimation of the PO_4^{3-} concentrations characteristic of temperate lakes in the summer epilimnion. The acidic molybdenum blue colorimetric method overestimates PO_4^{3-} by release and/or complexing with dissolved organic phosphorus compounds (Rigler 1966; Peters 1979; Tarapchak and Rubitschaun 1981; Cembella et al. 1984). Pettersson (1979) developed an enzymatic assay for estimating PO_4^{3-}, but the sensitivity of the fluorometric phosphatase substrates used is low, and the dissolved organic phosphorus pool may interfere (E. Bentzen and W. D. Taylor, in prep.). A preferred method is the bioassay developed by Rigler (1966) which uses small phosphate additions at the low end of the Michaelis–Menten curve to estimate the concentration of lake water PO_4^{3-} (e.g. Levine and Schindler 1980).

Fitting the Michaelis–Menten equation to phosphate uptake in lake water recently has been shown to be subject to several serious problems. Evaluation of the fit to uptake data collected from mixed planktonic assemblages often reveals serious lack of fit in different regions of substrate addition (Williams 1973; Brown et al. 1978; Tarapchak and Herche 1986, 1989). This poor fit is a consequence of the different values of V_m and K_t for different members of the mixed planktonic assemblage. This may be further aggravated by the use of an inadequate range of substrate concentrations in the experimental design. Another problem is the choice of fitting technique. A number of papers have dealt extensively with the statistical aspects of parameter estimation; the nature of the error and the fitting technique need to be considered in order to avoid bias in the parameter estimates (Endrenyi and Kwong 1972; Endrenyi 1974; Currie 1982; Raaijmakers 1987). A third problem when dealing with natural plankton is that the ambient concentration of available phosphate is usually unknown. Different amounts of PO_4^{3-} are added and the resulting substrate is the sum of the added PO_4^{3-} (P_a) and the ambient PO_4^{3-} (P_n). Although low substrate additions are used in the Rigler bioassay to define an upper limit to the possible P_n (Rigler 1966), Tarapchak and Herche (1988) recently demonstrated ambiguity in interpreting that upper bound.

This paper discusses these problems with particular reference to phosphate uptake by freshwater plankton communities. We demonstrate that the effect of the mixed community can be resolved by restricting either the size group of plankton or the substrate range. While the choice of fitting techniques has been discussed previously (Endrenyi 1974; Currie 1982; Ruppert et al. 1989), particular aspects of the determination of the velocity of phosphate uptake have not been explicitly addressed. The influence of a mixed community on the estimation of the uptake parameters, and the uncertainty of an unknown ambient PO_4^{3-}, places constraints on the choice of model used to estimate Michaelis–Menten parameters. We compare two nonlinear models with a commonly used linear transformation.

Comparisons of statistical models have relied upon simulated data (e.g. Currie 1982; Tarapchak and Herche 1988); our results are based entirely on data collected from freshwater epilimnetic plankton. We tested the models on water samples collected from Jacks Lake plus a sample from the enriched Bay of Quinte, where a different plankton assemblage and kinetics were expected. The uptake of $^{32}PO_4^{3-}$ was estimated in a standard kinetic design with additions of phosphate of up to 1500 nM. However, due to the importance of determining P_n and defining the low end of the curve, emphasis was placed on low concentrations. The models were compared by several goodness-of-fit evaluations. Thus, we offer useful guidelines on conducting and analyzing PO_4^{3-} uptake experiments, although these results are applicable to other nutrient uptake studies as well. We also mathematically define the maximum bound to the unknown ambient PO_4^{3-} as determined in the Rigler bioassay and show its relationship to the K_t of the smallest plankton size class.

Methods

Experiments were conducted with water from Sharpes Bay of Jacks Lake in the summers of 1988 and 1989. Jacks Lake is a mesotrophic lake with typical characteristics of P limitation (Lean and Pick 1981; Lean and White 1983). Additional samples were also taken from Brooks Bay of Jacks Lake (July 1989) and from the eutrophic Bay of Quinte, Lake Ontario, in August 1989 (Station C). All water samples were integrated through the epilimnion (0 – 6 m). Experimental vessels were soaked in acid, washed with phosphate-free detergent, and rinsed with deionized–distilled water.

TABLE 1. Total phosphorus (TP) concentration measured in the epilimnion, the number of uptake estimates performed in each assay, and the range of added PO_4^{3-} concentrations.

Site	Reference code	TP (nM)	n	Range of added PO_4^{3-} (nM)
Sharpes Bay				
June 23, 1988	J23	381[a]	13	1–240
June 29, 1988	J29	329[a]	12	1–120
Aug. 12, 1988	A12	239[a]	19	1–1500
Aug. 22, 1988	A22	429	14	2–150
Aug. 31, 1988	A31	290[a]	10	10–500
Sept. 23, 1988	S23	368	18	1–640
July 13, 1989	S89	320	23	0.5–1000
Brooks Bay				
July 12, 1989	B89	334	13	3–1000
Station C				
Aug. 22, 1989	StC	390	18	1–500

[a]TP data courtesy of D. Lean (NWRI, Burlington, Ont.).

Phosphate solutions were prepared with K_2HPO_4 by standard analytical techniques. Diluted standards were prepared only shortly in advance of the uptake experiments and were kept at 4°C or frozen until used. Phosphate was added from 0 up to 1500 nM (Table 1).

The uptake experiments were completed within 4–5 h of collecting the lake water. Subsamples of water (usually 40.0 mL) were measured with a graduated cylinder into polyethlyene beakers. Known amounts of PO_4^{3-} were added to the water subsample simultaneously with 10–20 kBq of carrier-free $^{32}PO_4^{3-}$ (ICN Biomedicals). Total ^{32}P radioactivity added was determined in 0.5- or 1.0-mL aliquots of unfiltered water. Uptake of the ^{32}P was determined by filtering 0.5- or 1.0-mL aliquots onto 0.2-μm polycarbonate filters (Poretics Corp.). With no added phosphate, or at low phosphate additions, uptake was assayed at five times from 0.5 to 4 min after ^{32}P addition; higher substrate additions required longer incubations of up to 25 min. Two to 4 h after ^{32}P addition, aliquots were filtered through 0.2- and 1-μm polycarbonate filters to partition the size fractions responsible for uptake. Filters were dissolved with 100 μL of a 1:10 mixture of monoethanolamine – methylene chloride. Ecolume cocktail (ICN Biomedicals) was added to all samples prior to determining ^{32}P by liquid scintillation counting (Beckman).

The uptake rate constant, k, for $^{32}PO_4^{3-}$ was estimated by least squares regression on the initial slope of the ln (%^{32}P) remaining in solution as a function of time (Lean 1973). At zero or low P_a, the uptake of ^{32}P frequently was extremely rapid and biphasic (Lean and Nalewajko 1979). As a consequence, the slope and intercept would be under estimated by the method described above. To accurately estimate k in these cases, a second-order polynomial was fit to the data, and k was taken as the first derivative at time = 0 (Currie and Kalff 1984a). The uptake rate constant for $^{32}PO_4^{3-}$ is equal to the slope coef-

TABLE 2. Symbols and abbreviations used in the text.

PO_4^{3-}	Phosphate
P_n	Ambient phosphate concentration (nM)
P_a	Added phosphate (nM)
k	Uptake rate constant (/min)
k_i	Uptake rate constant at *i*th P addition
v, v_i	Velocity of PO_4^{3-} uptake (nM/min)
V_m	Maximum velocity of PO_4^{3-} uptake
K_t	Michaelis–Menten half-saturation constant for P transport (nM)
TT_0	Turnover time of ambient PO_4^{3-}, measured with carrier-free $^{32}PO_4^{3-}$; $= k^{-1}$ at $P_a = 0$ (min)
TT	Turnover time of PO_4^{3-}, estimated from $(K_t + P_n)/V_m$
SS	Sums of squares (minimized) = sum of (estimated − observed)^2
MM	Michaelis–Menten model

ficient for the linear term. The data were first plotted to detect any nonlinearity in uptake as well any outliers (from experimental error). Any points which were clearly outliers in the linear plot were excluded from the calculation of k. The mean regression parameters obtained for 70 determinations of k were as follows: the mean r^2 was 0.994 (SD = 0.01); mean intercept was 99.6% (SD = 1.01); and the average standard error for the slope estimate was 9.3%. The reciprocal of k is the turnover time (TT) for PO_4^{3-}, which is the time required for an amount of P equivalent to the ambient pool to be taken up by the plankton and the same amount to be released into solution (Rigler 1973).

The uptake of ^{32}P by cells in the 0.2–1 μm size fraction was estimated by subtracting the ^{32}P radioactivity greater than 1 μm from the ^{32}P radioactivity retained on the 0.2-μm filter and expressing that remainder as a proportion of the ^{32}P activity retained on the 0.2-μm filter. The rate constant for P uptake by the 0.2–1 μm fraction is then k multiplied by that proportion.

Theory and Models

Addition of carrier-free $^{32}PO_4^{3-}$ to lake water does not alter the steady-state uptake dynamics of phosphate, and thus reflects the ambient uptake of PO_4^{3-} into cells (Rigler 1973). However, estimating the velocity (v) of P flux into cells requires knowledge of the ambient phosphate concentration. The uptake of $^{32}PO_4^{3-}$ yields k, which is multiplied by the PO_4^{3-} concentration to estimate v. When $^{32}PO_4^{3-}$ is added with known concentrations of $^{31}PO_4^{3-}$ (P_a), the concentration of $^{31}PO_4^{3-}$ (P_n) already present is unknown. Thus, the Michaelis–Menten Equation (1) is actually estimated as

$$(2)\quad v = k\cdot(P_n + P_a) = \frac{V_m\cdot(P_n + P_a)}{K_t + (P_n + P_a)}$$

(see Table 2 for a summary of the terms used). Since P_n is unknown, the model fits $k\cdot P_a = v_a$ against P_a. In P-limited systems, P_n is low in concentration and has a negligible effect on the estimation of V_m, but estimation of K_t is not possible because K_t cannot be distinguished from P_n. Therefore, the sum $(K_t + P_n)$ is generated by the nonlinear estimation. Michaelis–Menten (MM) curves for three representative experiments are shown in Fig. 1A, 1C, and 1E.

Papers which have addressed the statistical problems of estimating the Michaelis–Menten parameters V_m and K_t using different models have used simulated data with velocity as the dependent variable (Endrenyi 1974; Currie 1982). However, since the true velocity of uptake at $(P_a + P_n)$ cannot be determined, a better estimation of the parameters would fit the measured uptake rate constant, k_i, directly with each added P concentration (P_{ai}). Taking the reciprocal of Equation (2) after dividing each side by $(P_a + P_n)$ yields a linear expression of k^{-1}, which is the turnover time (TT), against P_a:

$$(3)\quad TT = \frac{(K_t + P_n)}{V_m} + \frac{P_a}{V_m}.$$

This equation was described by Wright and Hobbie (1965) and is analogous to the Woolf linear transformation of the Michaelis–Menten equation (Haldane 1957) but includes P_n with K_t. The reciprocal of the estimated slope is V_m, and $(K_t + P_n)$ is obtained by dividing the intercept by the slope. Examples of linearized plots are shown in Fig. 2A, 2C, and 2E.

Depending upon the nature of the error of k or v, it has been shown that such a linear transformation may severely bias the parameter estimates, particularly K_t (Endrenyi and Kwong 1972; Endrenyi 1974; Currie 1982; Li 1983; Ruppert et al. 1989). The most reliable parameter estimates are obtained by fitting a nonlinear model, such as Equation (1). An alternative nonlinear model directly fits k versus P_a after dividing each side of Equation (2) by $(K_t + P_n)$:

$$(4)\quad k_i = \frac{V_m}{(K_t + P_n) + P_{ai}}.$$

This equation avoids the problems caused by an unknown true P_n. Li (1983) used this form of the Michaelis–Menten equation for glucose uptake by plankton, so we will refer to Equation (4) as the Li equation to distinguish it from the MM nonlinear model. Examples of the Li plots are shown in Fig. 3A, 3C, and 3E.

Importance of Substrate Range and Plankton Size

The lack of fit to the Michaelis–Menten equation for glucose uptake by a natural plankton assemblage was already noted by Vaccaro and Jannasch (1966). With use of simulated data, Williams (1973) demonstrated that the complicated sum of Michaelis–Menten parameters for the component species caused poor fit for the whole community to the equation. Using the Woolf transformation, Williams showed distinct differences in fit in different substrate regions as significant variations in the slope of TT versus substrate. Tarapchak and Herche (1986) repeated these observations with PO_4^{3-} kinetics data from Lake Michigan and, also with the Woolf model, showed that extrapolating the line from TT at high substrate additions vastly overestimated observed TT at low substrate additions. We have compared the parameters estimated by the three models using a restricted substrate range ($P_a \leq 25$ nM) with estimates generated using all P_a.

Isolation of Michaelis–Menten parameters from the individual species in natural populations is very difficult, and the actual effect of the mixed assemblage on the mean community parameters is unclear. Williams (1973) and Tarapchak and Herche (1986) used simulated populations to demonstrate that the relative abundances of particular species influenced parameter estimates for the community. Smith and Kalff (1983) and Currie and Kalff (1984a) demonstrated that the smallest algae and bacteria often have a competitive advantage in PO_4^{3-} uptake. Characterization of uptake kinetics for different size fractions

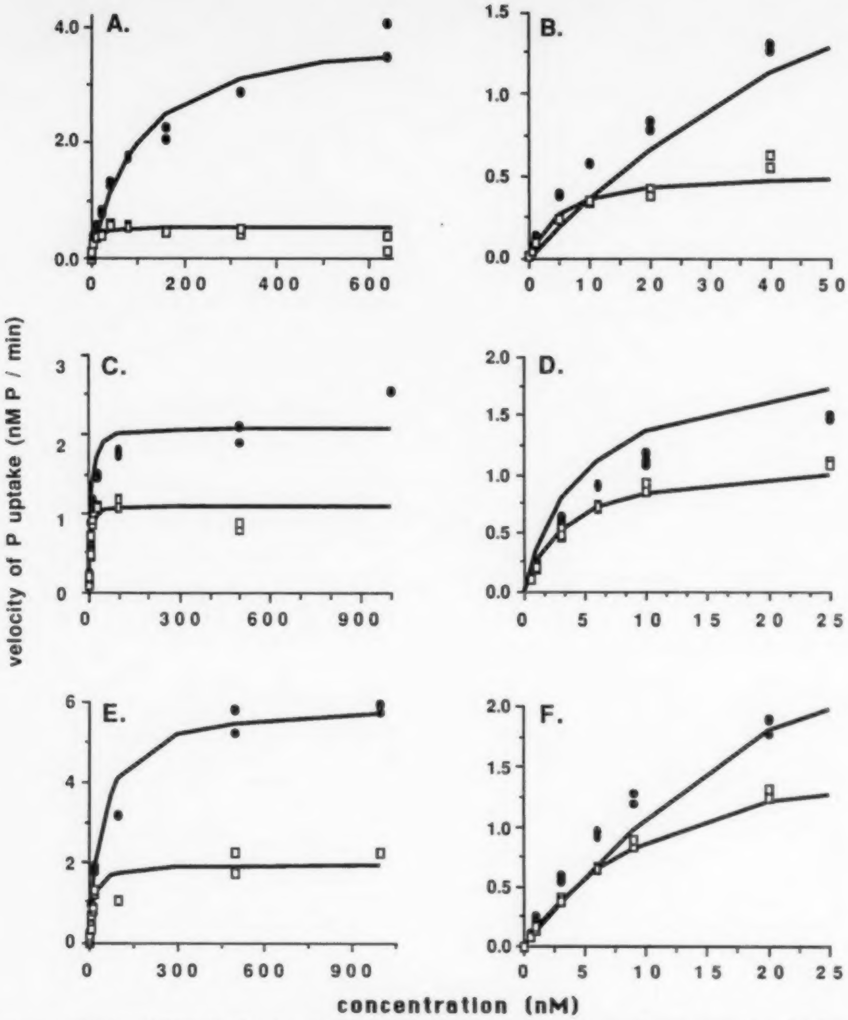

FIG. 1. Uptake of $^{32}PO_4^{3-}$ fitted to the Michaelis–Menten equation by nonlinear regression for three experiments: (A) Sharpes Bay, Sept. 23, 1988. (C) Sharpes Bay, July 1989, and (E) Brooks Bay, July 1989. Curves are fitted to the whole community (>0.2 μm, solid points) and to the 0.2–1 μm size fraction (open squares). B, D, and F are magnifications of the previous curves (A, C, and E, respectively) for the low-substrate region.

of plankton could help improve the estimation of the Michaelis–Menten parameters if size fractions of the plankton fit the model better than the whole community.

All three models thus were assessed on the 0.2–1 μm size fraction as well as on the whole community. The reason for focusing on this particular size fraction is that it commonly dominates phosphate uptake kinetics in P-limited systems (Faust and Correll 1976; Berman 1985; Currie and Kalff 1984b; Currie et al. 1986). The impact of the K_t for this size fraction on the whole community K_t is demonstrated. It will be shown below that this bears also on the determination of P_n in the Rigler bioassay.

Model Evaluation

Nonlinear and least squares regressions were computed using SYSTAT (Wilkinson 1988). The minimized nonlinear estimations were derivative-free Quasi-Newton. Goodness-of-fit to each of the models was assessed in three ways.

(i) A useful feature apparent in Equation (3) is that the intercept, $(K_t + P_n)/V_m$, is equivalent to the ambient turnover time for phosphate. Uptake rate constants for carrier-free $^{32}PO_4^{3-}$ were not used in fitting any of the models. Thus, an independent assessment of the goodness-of-fit is to compare $(K_t+P_n)/V_m$ generated by each model to the ambient $^{32}PO_4^{3-}$ turnover time. The sum of the squared differences (SS) between observed and estimated TT permits a direct comparison between the models over all experiments.

(ii) The F value for each fitted model was compared. Since this is the ratio of the sums of squares of the fitted model to the sum of squared residuals, a higher value for F indicates a reduction in the residuals. The same data are used to fit each model and thus the F for each model has the same degrees of freedom.

(iii) The percent standard errors (SE) associated with the individual parameter estimates, $(K_t + P_n)$ and V_m, can be com-

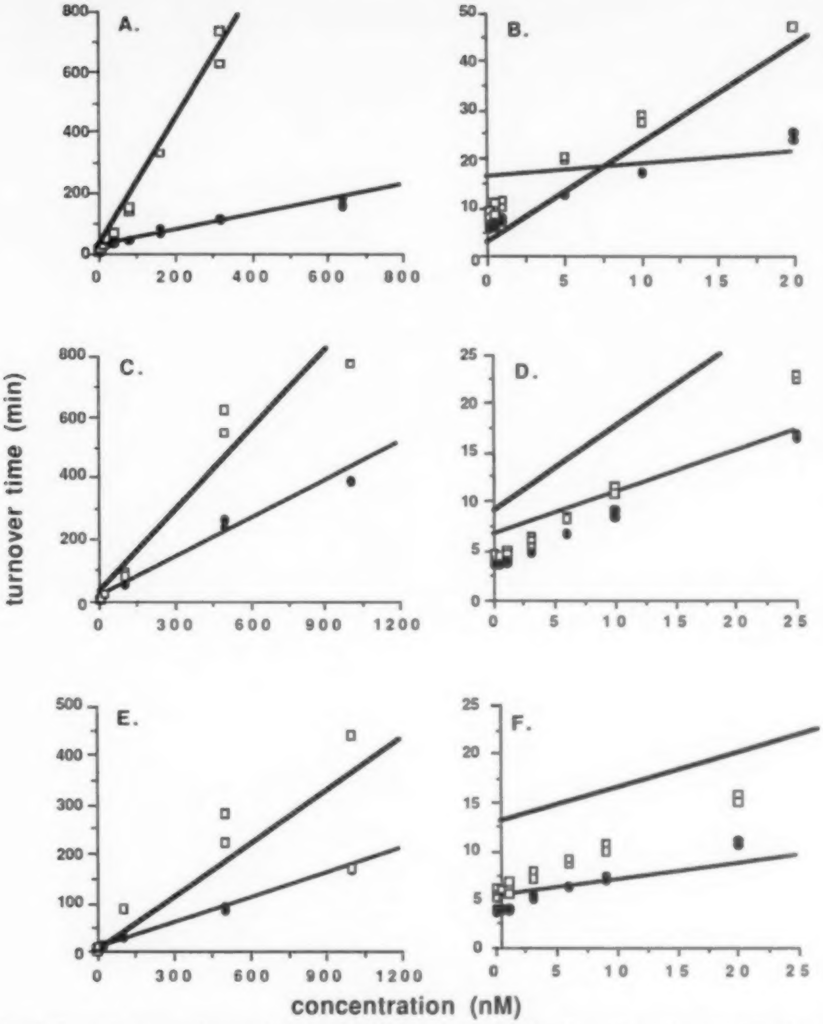

FIG. 2. Same three experiments shown in Fig. 1 fitted using the Woolf transformation over all substrate concentrations (A, C, E) and showing the magnified low-substrate region (B, D, F).

pared between the models. For the two nonlinear models, the SE are directly available from the fitting program. However, for the Woolf linearization, $(K_t + P_n)$ is estimated as the intercept (β_0) divided by the slope (β_1). It is thus necessary to calculate SE for the ratio of β_0/β_1. The estimates of $\{\beta_0, \beta_1\}$ express a significant degree of covariance, since K_t is equal to S at $V_m/2$, and this influences the estimate of the $SE\{\beta_0, \beta_1\}$:

$$(5) \quad \text{cov}\{\beta_0, \beta_1\} = r \cdot \text{SE}\{\beta_0\} \cdot \text{SE}\{\beta_1\}$$

where r is the correlation between $\{\beta_0, \beta_1\}$ and their SE are directly obtained from the linear regression. An approximation of the SE $\{\beta_0, \beta_1\}$ is estimated from the square root of the following expression (J. O'Hara-Hines, Department of Statistics, University of Waterloo, pers. comm.; developed from Fieller's Theorem, in Govindarajulu 1988):

$$(6) \quad \text{var}\{\beta_0/\beta_1\} = \frac{1}{\beta_1^2} \cdot \text{SE}\{\beta_0\}^2 + \frac{\beta_0^2}{\beta_1^4} \cdot \text{SE}\{\beta_1\}^2 - \frac{2\beta_0}{\beta_1^3} \cdot \text{COV}\{\beta_0, \beta_1\}.$$

In addition to the above comparisons, examination of the residuals for each fitted model demonstrates where problems of fit may exist (Endrenyi 1974; Li 1983). These evaluations were employed on whole community data and on the 0.2–1 μm size fraction. In addition, the models were also tested separately for $P_a \leq 25$ nM, as discussed above.

Results and Discussion

Model Choice

Uptake of PO_4^{3-} by the whole community (>0.2 μm) and by the 0.2–1 μm fraction fit reasonably well to the MM model (Fig. 1A, 1C and 1E) and very well to the Li model (Fig. 3A, 3C, and 3E). The Woolf transformation fit the data less well (Fig. 2A, 2C, and 2E). Magnification of the low substrate region (P_a < 40 nM) for each curve demonstrates clearly the lack of adherence of the whole community data (>0.2 μm) to the curves predicted by both the MM (Fig. 1B, 1D, and 1F) and Woolf models (Fig. 2B, 2D, and 2F). The Li model does

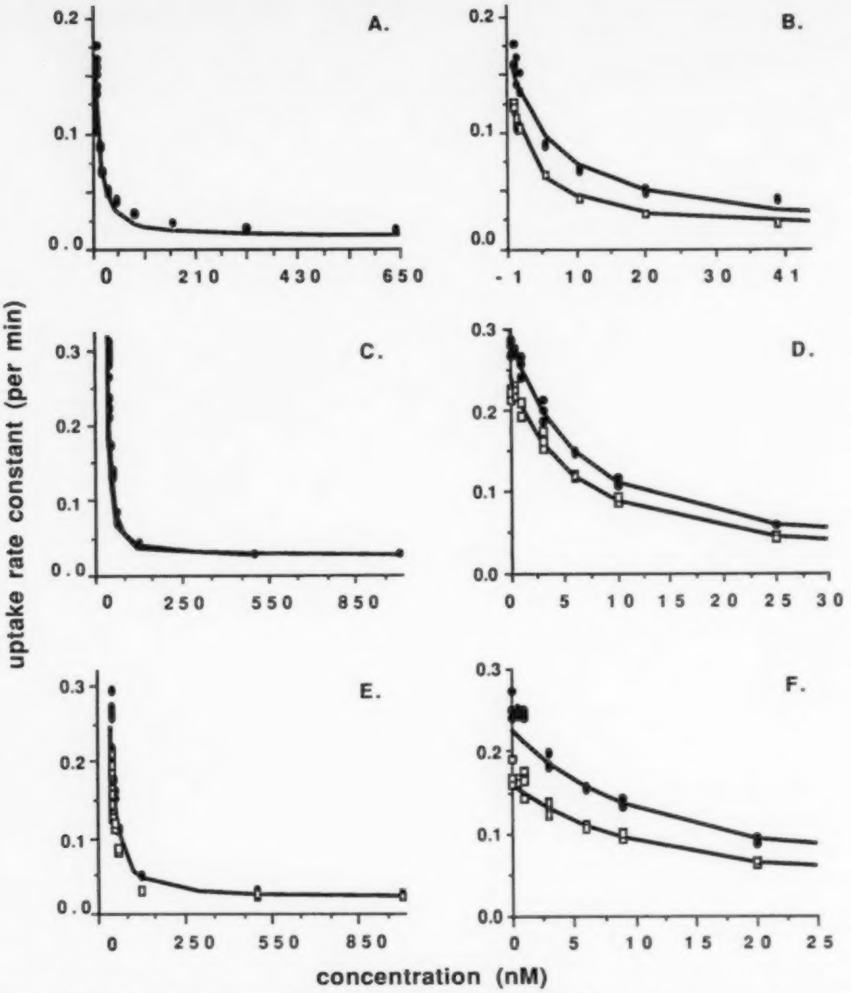

FIG. 3. Same three experiments shown in Fig. 1 and 2 fitted using the Li nonlinear model over all substrate concentrations (A, C, E) and showing the magnified low-substrate region (B, D, F). Predicted curves for the 0.2–1 μm size fraction in A, C, and E are not shown, since they are very similar to the whole-community curve (>0.2 μm); they are shown in the magnified plots (B, D, F).

not show this lack of fit at low substrate additions (Fig. 3B, 3D, and 3F).

The three model evaluation statistics supported these visual observations. The first was the comparison of the ratio of the estimated $(K_t + P_n):V_m$ to the ambient phosphate turnover time. Both the MM and the Woolf models seriously overestimated TT for the whole community when analyzed over the entire substrate range (Table 3). The Li model generated the lowest SS between estimated and observed TT.

When the models were fit using $P_a \leq 25$ nM, all three were more successful in estimating TT (Table 3) although the lowest SS was still obtained with the Li model. The difference in success between the MM and Li models was a consequence of a single experiment (StC) for which the MM model greatly overestimated TT. Removal of the StC result from the sums of squares lowered the SS from 240 to 11 for the MM model and from 24 to 10 for the Li model. The reason for the MM model's poor fit in StC is unknown and may be spurious; the MM model may normally perform well with low P_a data.

The improvement in TT estimates with all of the models for the $P_a \leq 25$ nM range can be understood by an analysis of the 0.2–1 μm data. At carrier-free additions of $^{32}PO_4^{3-}$, 70% or more of the uptake may be 0.2–1 μm. This high affinity by the small size fraction for phosphate is thought to be coupled with a low V_m (Lean and White 1983; Button 1985). In all of our examples, uptake was dominated by the 0.2–1 μm fraction and V_m typically was reached before 25 nM. Because the percent of uptake by this fraction remained constant until V_m was obtained, fitting the models at $P_a < 25$ nM was somewhat analogous to fitting the models on the 0.2–1 μm data. Both the MM and the Li models predicted the TT reasonably well for the 0.2–1 μm fraction, especially when only the $P_a < 25$ nM were considered. When all P_a were used, the fit of the Woolf model was poor; in several cases it generated negative intercepts and estimates of TT. Once again, StC inflated the SS for the MM model for $P_a < 25$ nM, and its removal reduced the MM model SS to 11 (Table 3).

All of the models resulted in high values of *F*, with significance levels less than 1% (Table 4). The Li model

TABLE 3. Comparison of estimates of $(K_t + P_a)/V_m$ with $^{32}PO_4^{3-}$ turnover time (TT_0) from the three models. Site codes are listed in Table 1. Models were fitted to either all P_a (full data) or to the subset of $P_a \leq 25$ nM for both the total community and the 0.2–1 μm size class. The SS were computed between estimated and observed TT; Aug. 31 data were not included in SS (see text). All units are minutes. MM = Michaelis–Menten model; nc = not computed.

	Full data				$P_a \leq 25$ nM		
Site	TT_0	MM	Woolf	Li	MM	Woolf	Li
Total							
J23	6.8	16.1	15.4	8.4	7.1	8.0	8.3
J29	5.1	13.4	10.6	4.1	6.2	5.6	4.1
A12	3.2	6.9	7.8	3.2	5.5	2.9	3.1
A22	4.0	7.8	7.4	6.9	5.7	6.0	6.3
A31	4.9	26.4	20.4	8.1	nc	nc	nc
S23	7.5	25.6	19.3	6.8	8.5	7.6	6.5
S89	3.6	2.5	7.0	3.4	3.5	3.5	3.4
B89	3.9	7.7	6.7	4.4	4.4	4.4	4.4
StC	23.9	33.1	32.2	28.4	39.0	28.4	27.6
SS		612	365	33	240	55	24
0.2–1 μm							
J23	8.4	7.5	5.6	8.2	7.4	7.9	7.9
J29	6.8	6.8	2.6	4.1	6.4	6.1	5.4
A12	4.8	2.7	−57.7	3.8	7.1	5.8	3.8
A22	6.2	8.5	6.4	8.9	8.1	7.9	8.6
A31	8.2	−3.1	−663	−3.6	nc	nc	nc
S23	10.6	9.3	−0.6	8.7	9.6	9.2	8.6
S89	4.6	3.1	9.7	4.1	3.9	4.0	4.1
B89	5.8	6.3	11.8	6.2	6.3	6.3	6.3
StC	39.6	44.9	7.1	40.3	81.5	49.1	40.1
SS		43	5175	20	1767	97	14

TABLE 4. Goodness-of-fit of the three models compared by the *F* statistic. The models were run as described for Table 3. The r^2 for the Woolf regressions are also given. Significance levels associated with the *F* statistic were less than 0.01. nc = not computed.

	Full data			$P_a \leq 25$ nM			Woolf r^2	
Site	MM	Woolf	Li	MM	Woolf	Li	Full	$P_a \leq 25$ nM
Total								
J23	215	199	2617	534	553	2478	0.943	0.993
J29	226	142	947	647	296	970	0.928	0.977
A12	96	62	459	230	21	311	0.771	0.688
A22	482	568	747	335	68	1327	0.978	0.905
A31	117	72	686	nc	nc	nc	0.888	0.972
S23	572	413	536	1648	283	745	0.960	0.976
S89	546	1112	9226	8892	275	7034	0.981	0.997
B89	648	3626	3626	3682	537	2825	0.997	0.985
StC	1729	3984	514	1028	35	449	0.996	0.760
0.2–1 μm								
J23	492	372	2228	1146	465	697	0.971	0.994
J29	96	39	2095	366	219	1313	0.790	0.973
A12	51	116	651	170	34	329	0.885	0.803
A22	290	952	1392	407	106	1228	0.987	0.937
A31	142	180	191	nc	nc	nc	0.952	nc
S23	401	1370	1454	1531	636	885	0.989	0.989
S89	548	375	6430	7923	5544	4932	0.947	0.997
B89	160	597	2268	3387	519	2302	0.980	0.985
StC	112	939	296	203	22	221	0.989	0.750

obtained the highest values of *F* for the whole community when all P_a were used. However, differences in performance were again diminished with use of the restricted substrate range. Both nonlinear models were superior to the Woolf model in most cases. Nonetheless, the r^2 values for the Woolf regressions were greater than 0.93 in most instances (Table 4) which would lend a false impression that the Woolf model was a good fit. The Li model consistently generated the highest F values using the 0.2–1 μm subgroup, although by restricting $P_a < 25$ nM the MM model sometimes had the greater F.

The SE for $(K_t + P_n)$ for these three models showed that better fits were obtained with the Li model than with either the MM or the Woolf model (Table 5). The $(K_t + P_n)$ estimates from the Li model for the whole community, using both the full and the restricted substrate data, were also much lower than those for Woolf and MM models. While the differences between the MM and Li models were reduced with the focus upon the 0.2–1 μm group, the SE for the Li model were still lower. The $(K_t + P_n)$ estimates by the Woolf model for the 0.2–1 μm group were often not reliable, with both negative estimates and SE > 100%. This was due to the large error often associated with the intercept in the Woolf regression.

Estimates of V_m among the three models were fairly similar to each other (Table 6), although for the whole community and using the full data the Li estimates tended to be lower. The percent SE were not always lower for the Li model. Restricting the P_a to less than 25 nM and focusing upon the 0.2–1 μm subfraction generated similar V_m and SE by all three models. Using simulated data, Endrenyi (1974) and Currie (1982) also observed that estimates of V_m were less subject to bias by different models than the estimates of K_t.

Substrate Range

Currie (1982) discussed the importance of the experimental design for uptake studies, including the range and spacing of substrate concentrations. Currie recommended geometrical spacing of added substrates; in addition, an adequate range of substrate concentrations is clearly important. If low P_a are not assayed, an inaccurate estimate of $(K_t + P_n)$ is highly probable. The $(K_t + P_n)$ for five of the nine experiments were less than 10 nM (by the Li model). Unless at least one of the P_a is less than 10, $(K_t + P_n)$ may not be accurately ascertained. We tested the effect of substrate range on the estimation of the parameters by running the models with different lower bounds on P_a. The effect of excluding P_a less than 10 or 20 nM generated an increased estimate of $(K_t + P_n)$ in several cases and analysis of the 0.2–1 μm size fraction was not always possible. Since the percent of uptake attributable to this fraction is reduced at higher P_a, the activity of this fraction is inadequately represented and the estimate of $(K_t + P_n)$ was negative. On August 31 (A31), the lowest P_a were 10 and 25 nM and it was not possible to assess the $(K_t + P_n)$ of the 0.2–1 μm size fraction (Table 5). As uptake at low substrate concentrations is dominated by this size fraction, the validity of the parameter estimates for this particular experiment is in doubt, even for the whole community (>0.2 μm). When the uptake of P is very rapid (TT < 10 min), one or two P_a less than 10 nM should be included. High P_a are necessary when the desired parameter is the maximum value for the community V_m.

Rigler Bioassay and Estimation of P_n

The Rigler bioassay uses estimates of k_i for a series of low P_{ai}; the range of P_{ai} used in these experiments was 0–25 nM. The k_i for each P_{ai} are multiplied by the sum of P_{ai} plus an assumed P_n. This exercise is repeated for a suite of assumed P_n. The resultant sets of v_i are plotted against the sum of $(P_{ai} + P_n)$; a sample plot is shown in Fig. 4. Velocity of uptake as described by the Michaelis–Menten equation is a positive and decelerating function of the P concentration. Since $v = 0$ at zero substrate, the assumed P_n should maintain the curve in a downward direction towards the origin. Assuming a P_n which is too high will create an upward deflection in the vicinity of the origin. The Rigler estimate of P_n is that which, when exceeded, will cause this upswing in the curve (Rigler 1966). It is an upper limit to the true P_n.

The S89 data are replotted in Fig. 4 with curves for assumed P_n of 0, 4.8, 6.5, and 8 nM. A curve with zero slope is evident

TABLE 5. Estimate of $(K_t + P_n)$ obtained with the three models for the whole community and for the 0.2–1 μm size fraction using all substrate additions (full data) and $P_a \leq 25$ nM. nc = not computed.

Site	Full data			$P_a \leq 25$ nM		
	MM (%SE)	Li (%SE)	Woolf (%SE)	MM (%SE)	Li (%SE)	Woolf (%SE)
Total						
J23	22.2 (37.3)	8.4 (5.6)	21.1 (3.9)	6.4 (11.9)	7.9 (7.4)	7.3 (7.1)
J29	28.5 (34.5)	3.6 (11.6)	21.5 (27.4)	7.2 (19.9)	3.5 (11.6)	6.3 (17.4)
A12	49.2 (37.2)	8.3 (19.8)	66.9 (>100)	32.6 (50.9)	7.2 (24.2)	16.7 (36.0)
A22	26.0 (16.9)	18.7 (16.1)	24.0 (13.9)	9.8 (27.8)	12.4 (14.2)	9.9 (22.9)
A31	171.5 (36.4)	15.6 (19.3)	119.0 (31.2)	nc	nc	nc
S23	103.6 (17.6)	7.3 (15.5)	76.4 (19.1)	10.3 (14.2)	5.9 (13.0)	8.5 (13.2)
S89	5.3 (17.0)	6.2 (4.1)	16.7 (48.5)	6.5 (4.5)	6.2 (4.7)	6.4 (3.2)
B89	45.9 (17.2)	13.8 (9.2)	40.7 (17.4)	13.6 (8.3)	13.3 (10.7)	13.5 (7.4)
StC	38.7 (7.2)	20.2 (22.7)	36.3 (8.7)	64.2 (35.4)	17.3 (22.6)	26.4 (23.1)
0.2–1 μm						
J23	4.4 (25.8)	4.9 (11.6)	3.2 (>100)	4.4 (21.7)	4.6 (27.9)	4.6 (10.8)
J29	4.6 (54.8)	3.2 (8.3)	1.5 (>100)	4.2 (27.6)	3.2 (10.7)	4.0 (29.9)
A12	2.7 (64.7)	4.3 (14.4)	−25.1	14.3 (44.6)	4.3 (20.7)	10.1 (37.6)
A22	8.5 (23.0)	8.9 (11.1)	5.8 (37.3)	7.1 (24.3)	8.2 (13.7)	7.6 (22.8)
A31	−1.3	−1.4	−39	nc	nc	nc
S23	5.0 (24.1)	4.2 (8.9)	−0.3	4.8 (13.6)	4.0 (11.9)	4.6 (13.2)
S89	3.4 (15.3)	5.8 (4.8)	10.8 (>100)	5.2 (4.7)	5.9 (5.6)	5.3 (3.5)
B89	12.4 (25.7)	13.0 (10.4)	26.2 (55.4)	13.6 (8.1)	13.7 (11.7)	13.6 (7.5)
StC	11.6 (32.5)	8.5 (19.4)	1.4 (>100)	52.1 (>100)	8.3 (23.3)	13.4 (39.4)

TABLE 6. Estimates of V_m (nM/min) obtained with the three models for the whole community and for the 0.2–1 μm size fraction, using all substrate additions (full data) and $P_a \leq 25$ nM. nc = not computed.

Site	Full data			$P_a \leq 25$ nM		
	V_m (%SE)	V_m Li (%SE)	V_m Woolf (%SE)	V_m MM (%SE)	V_m Li (%SE)	V_m Woolf (%SE)
Total						
J23	1.4 (9.3)	1.0 (4.3)	1.4 (7.1)	0.9 (7.7)	0.9 (5.4)	0.9 (4.3)
J29	2.1 (12.5)	0.9 (8.0)	2.0 (8.4)	1.2 (7.4)	0.8 (7.9)	1.1 (5.8)
A12	7.1 (9.4)	2.6 (14.7)	6.4 (12.7)	6.0 (32.9)	2.4 (17.8)	4.0 (22.0)
A22	3.3 (6.3)	2.7 (12.1)	3.2 (4.2)	1.7 (14.0)	2.0 (10.4)	1.7 (12.1)
A31	6.5 (15.1)	2.1 (10.1)	5.8 (11.8)	nc	nc	nc
S23	4.1 (5.9)	1.1 (11.3)	4.0 (4.9)	1.2 (6.5)	0.9 (9.4)	1.1 (5.9)
S89	2.1 (4.1)	1.8 (3.2)	2.4 (3.0)	1.9 (1.9)	1.8 (3.8)	1.9 (1.4)
B89	6.0 (3.6)	3.1 (6.7)	6.1 (1.6)	3.1 (4.5)	3.1 (7.7)	3.1 (4.3)
StC	1.2 (2.4)	0.7 (18.1)	1.1 (1.6)	1.6 (28.6)	0.6 (17.8)	0.8 (16.8)
0.2–1 μm						
J23	0.6 (4.8)	0.6 (5.2)	0.6 (5.2)	0.6 (4.8)	0.6 (12.5)	0.6 (4.6)
J29	0.7 (10.8)	0.6 (5.7)	0.6 (16.1)	0.7 (7.8)	0.6 (7.4)	0.6 (6.8)
A12	1.0 (11.8)	1.1 (9.9)	0.4 (9.3)	2.0 (21.7)	1.1 (14.3)	1.7 (17.2)
A22	1.0 (6.4)	1.0 (7.4)	0.9 (3.2)	0.9 (10.8)	0.9 (9.2)	0.8 (9.7)
A31	0.4 (25.6)	0.4 (21.0)	0.1 (7.5)	nc	nc	nc
S23	0.5 (4.6)	0.5 (6.2)	0.5 (2.7)	0.5 (4.5)	0.5 (8.3)	0.5 (4.0)
S89	1.1 (3.9)	1.4 (3.8)	1.1 (5.2)	1.3 (1.8)	1.4 (4.4)	1.3 (1.3)
B89	2.0 (7.1)	2.1 (7.4)	2.2 (4.1)	2.2 (4.4)	2.2 (8.5)	2.2 (4.4)
StC	0.3 (9.7)	0.2 (14.8)	0.2 (3.3)	0.6 (125)	0.2 (17.8)	0.3 (21.5)

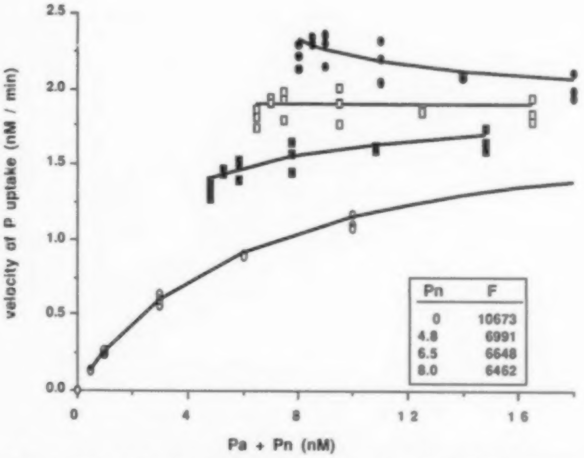

FIG. 4. Low end of the S89 curve as used in the Rigler bioassay. Assumed P_n of 0, 4.8, 6.5, and 8 nM were used in Equation (2) and the resultant v_i are plotted against the sum of P_a and the assumed P_n. The actual data are shown together with the predicted curve using V_m = 1.9 nM/min and $(K_t + P_n)$ = 6.5 nM (see text). The *F* values for the curves fit with each of the assumed P_n are given.

at an assumed P_n of 6.5 nM; thus, any higher value will cause the upswing. The true P_n could be any value less than 6.5 nM. A literal interpretation of the Rigler maximum P_n would be a value which would allow the curve its downward concavity towards the origin. Visual inspection of curves as a means of choosing P_n based upon these criteria may lead to a somewhat arbitrary decision about P_n. Tarapchak and Herche (1988) noted that this protocol is unsatisfactory because the substrate region less than the lowest $(P_a + P_n)$ is nonobservable and the actual shape of the curve may take a number of forms.

TABLE 7. Estimates of the Rigler P_n using Equation (7). The P_a bordering the slope segments for which P_n was found are given.

Site	Total data P_n (nM)	0.2–1 μm P_n (nM)	P_a segments
J23	3.0	2.7	1, 5, 10
J29	2.3	1.4	1, 5, 10
A12	1.9	0.7	1, 3, 6
A22	0.6	0.9	0.8, 2, 4.5
S23	3.1	2.1	1, 5, 10
S89	4.8	2.1	0.5, 1, 3
B89	6.0	10.2	3, 6, 9
StC	2.8	nc	1, 3, 6

Currie (1986) presented a simple mathematical formulation for estimating the Rigler maximum P_n based upon the comparison of slopes connecting adjacent estimates of v_i in the low substrate region of the MM plot. The Rigler maximum P_n is the minimum assumed P_n causing two adjacent slope segments to become equal when inspected along all the segments at the low end of the MM curve. Exceeding this assumed P_n would cause a positive inflection along some part of the low end of the curve. This P concentration is found as follows (Currie 1986):

$$(7) \quad P = \frac{B\,(k_i x_i - k_{i-1} x_{i-1}) - k_{i-1} x_{i-1} + k_{i-2} x_{i-2}}{k_{i-1} - k_{i-2} - B\,(k_i - k_{i-1})}$$

where the k_i are the uptake rate constants at each phosphate addition, here represented by x_i rather than P_{ai} for simplicity. The factor $B = (x_{i-1} - x_{i-2})/(x_i - x_{i-1})$. (Note that the denominator of Equation (7) had a printing error in the original paper.) Equation (7) is repeated for each pair of adjacent slope segments and the lowest resultant P obtained is chosen (Currie 1986).

The P_n estimated by Equation (7) ranged from 0.6 to 10.2 nM (Table 7); these values were similar to those reported elsewhere for P-limited systems (Brown et al. 1978; Levine and Schindler 1980; Tarapchak and Rubitschaun 1981; Currie 1986; Levine et al. 1986). The 0.2–1 μm size fraction usually generated a lower estimate of P_n. The concentrations of P_a bounding the slope segments over which P_n was estimated are also indicated (Table 7).

One problem with Equation (7) which has not been dealt with is the effect of experimental error on the individual estimates of k_i at each P_{ai}. Random error in the range of only 5% for a given k_i will seriously affect the estimate of any single slope segment and thus jeopardize the estimate of P_n. For example, if the slope segments from 1 to 5 and 5 to 10 nM added phosphate had error-free k_{i-2}, k_{i-1}, and k_i of 0.106, 0.0784, and 0.0591 for x_{i-2}, x_{i-1}, and x_i of 1, 5, and 10 nM, respectively, then $B = 0.80$ and $P = 10.2$. However, if the error for k_{i-2} is +5%, for example, k_{i-2} would be 0.1113 and P would be estimated as 6.96, a difference of 32%. Thus even small but realistic errors in the k_i are serious, especially since their real magnitude is unknown. Another problem with Equation (7) is the choice of substrate range; the slopes of adjacent segments will be a function of the distance between the x_i used in the bioassay. A large spacing between x_i would not be very sensitive to a small P_n.

Elimination of the unknown variability in the k_i might be achieved once the best model for estimating the Michaelis–Menten parameters has been selected. The parameters V_m and $(K_t + P_n)$ can then be used to generate predicted k_i using Equation (4). Estimations of P_n with Equation (7) using real and predicted k_i were compared.

When predicted k_i are used in Equation (7), the resulting estimates of P_n are equal to the $(K_t + P_n)$ which had been used to generate those k_i. In the absence of error in individual k_i, adjacent slope segments are equal only when the slope is zero and the v_i equal V_m. This is apparent in the S89 example (Fig. 4). The parameter estimates for $P_a \leq 25$ nM for the total community were $V_m = 1.9$ nM/min (Table 6) and $(K_t + P_n) = 6.5$ (Table 5; the Li and MM estimates were similar). Equation (7) estimated the Rigler P_n to be 4.8 based upon segments bounded by P_a of 0.5, 1, and 3 nM (Table 7). However, the predicted k_i produced segments with equal slopes at P_n equal to 6.5 where the slope is zero. Predicted k_i multiplied by the sum of P_a plus $(K_t + P_n)$ equals V_m. Any assumed P_n exceeding $(K_t + P_n)$ would cause the curve to swing upwards.

Using simulated data (which are error free) for a single-species population, Tarapchak and Herche (1988) observed the Rigler P_n to equal the K_t for that species. For a simulated mixed population, the Rigler maximum P_n equaled the smallest K_t in the mixture. Tarapchak and Herche (1988) concluded that the true resolution of the Rigler maximum P_n requires knowledge of the lowest value for K_t within the sampled population, which we show is obtained by using the Li model and isolating the 0.2–1 μm fraction.

When used on error-free data, Equation (7) cannot separate the sum of $(K_t + P_n)$. The accuracy of Equation (7) on real data is suspect due to the sensitivity of individual k_i to small degrees of error, although the estimated Rigler P_n are in the same range as the $(K_t + P_n)$ for the 0.2–1 μm fraction.

If the MM model (Equation (2)) is inferior to the Li model because of inaccurate estimates of v_i without knowledge of the true total substrate, then refitting the MM model for different assumed values of P_n may improve the goodness-of-fit and permit a solution to P_n. We tried this, and found that any addition of P_n to P_a decreased the *F* value from that obtained assuming zero P_a, even when the assumed P_n were much less than 1 nM. The decreased *F* values are apparent from increased residuals in observed relative to predicted data, as seen in the S89 example (Fig. 4). We conclude from this that most of the value of $(K_t + P_n)$ is actually K_t and that P_n is maintained at extremely low levels in P-limited systems.

Conclusions

Based upon the statistical comparisons, the Li model (Equation (4)) is the best choice for estimating the Michaelis–Menten parameters $(K_t + P_n)$ and V_m, and fitting the model requires neither an estimate of P_n nor a transformation of the measured variables. It yields sensitive estimates of the parameters despite the effect of a heterogenous assemblage, although occasionally the whole community V_m may be underestimated. In P-limited systems, analyzing the 0.2–1 μm fraction yields the lowest estimates of $(K_t + P_n)$. The worst choice for estimating the parameters is the linearized Woolf tansformation, particularily for $(K_t + P_n)$. This is in agreement with the earlier simulation studies of Endrenyi (1974) and Currie (1982), and yet the Woolf model is still used.

Ratkowsky (1986) proposed an alternative nonlinear estimation of the Michaelis–Menten parameters by putting both parameters into the denominator, claiming that the estimates are less biased than those from the standard Michaelis–Menten equation. This is achieved by dividing both the numerator and the denominator of either the Michaelis–Menten or the Li equation by $1/V_m$; the model produces the estimates $1/V_m$ and $(K_t + P_n)/V_m$. The results of this analysis were identical to those of the Li model when *k* was fitted against P_a, and thus offered no advantage. Another recent analysis by Choquet et al. (1987) suggested that the linearized model performed nearly as well as the Li model when the data were weighted, which is necessary when the error structure is heteroscedastic. With small data sets, the nature of the error structure is not always easy to establish. However, the Li model performed well both on the entire P_a range and on low P_a where no heteroscedasticity was apparent. An advantage in using a carrier-free tracer such as $^{32}PO_4^{3-}$ is that the ratio of the parameter estimates is comparable with carrier-free turnover times, and these results supported the fit by the Li model. Weighting does not seem to be justified and use of the simpler model is preferred.

Accurate estimation of the parameters does require additions of substrate in the same range as the $(K_t + P_n)$ or lower. In P-limited systems, the dominance of the smallest size fraction for P uptake has a bearing on the magnitude of the estimate of $(K_t + P_n)$. Since this fraction may also reach V_m at a low concentration of substrate, the necessity of sufficiently low P_a is demonstrated.

The Rigler maximum P_n was shown to be equal to the lowest estimate of $(K_t + P_n)$ generated for the 0.2–1 μm fraction, and resolution of K_t from P_n does not yet seem possible. The evidence suggests that the concentration of P_n is generally much lower than the sum of $(K_t + P_n)$. Literature values of P_n based upon the Rigler bioassay for P-limited lakes are of similar magnitude to the low $(K_t + P_n)$ reported here, although their precision might be questioned, since they were estimated either by subjective means or by reliance on individual data points. The results are similar because the bioassay uses low-substrate data. This suggests that low $(K_t + P_n)$ are to be expected in lake water of rapid PO_4^{3-} turnover time. The observation of a low Rigler estimate, but a higher value for the community K_t, has been made elsewhere and is a consequence of using low substrate additions for the P_n estimate and fitting the Michaelis–Menten equation to a broader range of P_a to estimate K_t. Therefore, this K_t estimate is influenced by cells with larger V_m and K_t, while the Rigler maximum P_n estimate is influenced by smaller cells with lower V_m and K_t.

Acknowledgments

We are much indebted to David Lean for use of the facilities at the Jacks Lake Field Station and for some of the total phosphorus data for Sharpes Bay. We also thank Scott Millard for the opportunity to sample in the Bay of Quinte. Ralph Smith, Robert Gensemer, and the two reviewers provided helpful criticisms to this manuscript. Funds were provided by Natural Sciences and Engineering Research Council of Canada (NSERC) and University of Waterloo scholarships to E.B. and by NSERC operating funds to W.D.T.

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Temporal Relation between Soluble Reactive Phosphorus and Factors in Stream Water and Sediments in Hoxie Gorge Creek, New York[1]

R. L. Klotz

Department of Biological Sciences, State University of New York, Cortland, NY 13045, USA

Klotz, R. L. 1991. Temporal relation between soluble reactive phosphorus and factors in stream water and sediments in Hoxie Gorge Creek, New York. Can. J. Fish. Aquat. Sci. 48: 84–90.

Temporal changes in soluble reactive phosphorus (SRP) were associated with sediment and streamwater factors in Hoxie Gorge Creek. Over the annual cycle, streamwater SRP was positively correlated with the zero equilibrium phosphate concentration (EPC_0) of the sediments at two disparate sites. Phosphate release during biological decomposition of organic matter in the sediments may have been an important source of SRP at times of high temperature and low discharge at an upstream site. At a downstream site, with lower sediment organic content and ATP, geochemical processes in the sediments as influenced by Ca may have been partly responsible for long-term SRP variations. Short-term SRP changes measured during a storm event were linked by field and laboratory analyses to variation in streamwater Ca. During the storm, streamwater Ca and SRP were negatively correlated ($r = -0.791$; $p < 0.05$). Laboratory sorption experiments showed that EPC_0 increased by 3.1 $\mu g \cdot L^{-1}$ as Ca decreased by 4 $mg \cdot L^{-1}$, simulating the change found during the storm. When streamwater Ca was experimentally increased by spiking with $CaCl_2$ in the field, SRP decreased. Some temporal changes in streamwater SRP can be explained by interactions between sediments and streamwater Ca.

Les modifications temporelles du phosphore réactif soluble (PRS) ont été liées aux facteurs relatifs aux sédiments et à l'eau de courant dans le ruisseau Hoxie Gorge. Au cours du cycle annuel, le PRS de l'eau de courant était corrélé de façon positive avec la concentration de phosphate à l'état d'équilibre dynamique (CPE_0) dans les sédiments à deux endroits distincts. La libération de phosphate au cours de la décomposition biologique de la matière organique dans les sédiments peut avoir été une source importante de PRS lorsque la température était élevée et la décharge faible dans un site en amont. Dans un site en aval, où le contenu des sédiments en matières organiques et en ATP est plus faible, les processus géochimiques se déroulant dans les sédiments et qui sont sensibles à l'action du Ca peuvent avoir été partiellement responsables des modifications à long terme du PRS. Les modifications de PRS à court terme mesurées dans des conditions de tempête ont été liées, au moyen d'analyses effectuées sur le terrain et en laboratoire, à la fluctuation du Ca dans l'eau de courant. Au cours de la tempête, le Ca de l'eau de courant et le PRS étaient corrélés de manière négative ($r = 0{,}791$; $p < 0{,}05$). Les expériences de sorption réalisées en laboratoire ont montré que le CPE_0 a augmenté de 3,1 $\mu g \cdot L^{-1}$ pendant que le Ca diminuait de 4 $mg \cdot L^{-1}$, ce qui constitue une simulation de la fluctuation observée au cours de la tempête. Lorsque le Ca de l'eau de courant a été augmenté de façon expérimentale par dopage avec du $CaCl_2$ sur le terrain, le PRS a diminué. Certaines modifications temporelles du PRS de l'eau de courant peuvent s'expliquer par les interactions entre les sédiments et le Ca de l'eau de courant.

Received December 11, 1989
Accepted July 23, 1990
(JA399)

Reçu le 11 décembre 1989
Accepté le 23 juillet 1990

A determination of factors regulating the supply of limiting nutrients is necessary for understanding production in aquatic systems. Phosphorus is considered the element which most commonly limits freshwater biological productivity (Wetzel 1983). While phosphorus (*P*) limitation has been better documented for lakes, there is evidence for P limitation in flowing waters (Stockner and Shortreed 1978; Elwood et al. 1981). Because P is a highly particle reactive element, it is not surprising that some lotic ecologists have concentrated on P interactions with sediments to determine what controls its rate of supply. They have found that phosphate is taken up by and released from particles through a complex series of geochemical "sorption" reactions known as the phosphate buffer mechanism (Froelich 1988). This phenomenon is important for the aquatic sciences because it has been linked to the maintenance of the phosphate concentration of rivers, streams, and estuaries at nearly constant values (Gessner 1960; Pomeroy et al. 1965; Taylor and Kunishi 1971; Meyer 1979; Klotz 1988). The mechanism provides for an often large and potentially available reservoir of P to replenish that removed from solution by aquatic organisms (Froelich 1988). Conversely, inputs of phosphate to the water column can be taken up by the sediments, resulting in reduced concentrations. Also, variation in sediment geochemistry can lead to differences in the dissolved P concentration between stream sites (Klotz 1988), which in turn may affect production.

Generalized terminology for phosphate buffer experiments was established by Froelich (1988). The zero equilibrium phosphate concentration (EPC_0), previously referred to as the "equilibrium phosphate concentration" (EPC; e.g. Taylor and

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[1] Contribution No. 20 of the Hoxie Gorge Field Station.

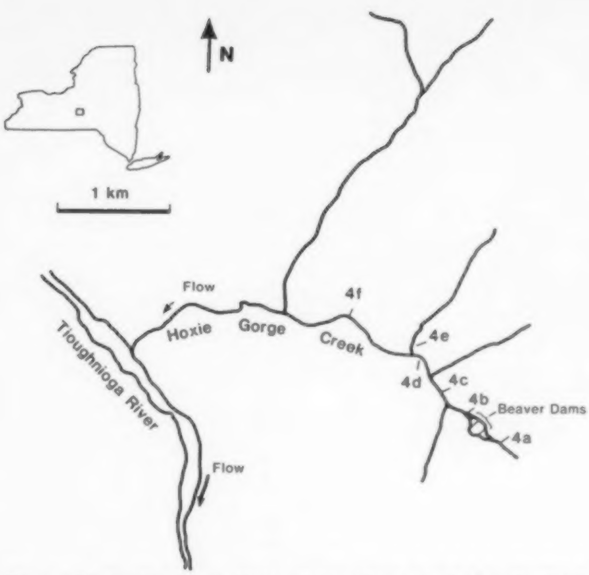

FIG. 1. Study sites along Hoxie Gorge Creek located in Cortland County, New York, USA.

Kunishi 1971; Meyer 1979; Klotz 1988), is the phosphate concentration in solution at which no net sorption nor desorption of phosphate by sediments occurs at any time during the incubation period. EPC_0 represents the P concentration at which the sediments display the maximum capacity for buffering solution P.

Studies on the phosphate buffer mechanism in rivers and streams have concentrated on the interactions between sediments and P at one point in time. These studies showed that sediments rapidly sorbed added P and indicated that sediments may regulate the dissolved P concentration, a conclusion based on the close agreement between streamwater dissolved P and EPC_0 (Gessner 1960; Taylor and Kunishi 1971; Meyer 1979). In Hoxie Gorge Creek, soluble reactive phosphorus (SRP) increased downstream and was highly correlated ($p < 0.01$) with the EPC_0 of the sediments along the downstream gradient (Klotz 1988). EPC_0 was believed to be regulated by a mechanism involving exchangeable Al on the sediments that was influenced by the ionic strength and specific cations (most importantly Ca^{2+}) of the solution.

Observations prior to this study indicated that both long-term (annual cycle) and short-term (storm event) variation in streamwater SRP occurred at Hoxie Gorge Creek in addition to the downstream increase noted above. The current study was done to determine whether these temporal changes in SRP were also correlated with EPC_0. Factors in the stream water and the sediments were assessed to determine which influenced SRP and EPC_0 temporally.

Study Area

Hoxie Gorge Creek is located in Cortland County in central New York State (Fig. 1). The stream was shaded throughout its length, limiting primary production to areas with an open canopy or to suitable times of year when the trees were bare. The watershed area for the length of the stream under study was 17.6 km^2 and was 94% wooded with the remainder primarily old field. Site 4a was surrounded by 2–8% slopes with poorly drained, strongly acid soils with fragipan at depths between 20 and 35 cm (USDA 1961). The stream channel at site 4a was about 1 m wide and the bottom consisted primarily of fine-grained sediments with a capacity to sorb large amounts of P (Klotz 1988). Sites 4d and 4f were located in the gorge area with walls of 25–55% slope, well-drained soils, and a depth to bedrock generally between 0.8 and 1.0 m (USDA 1961). Discharge at site 4f was determined to be 0.10 (0.23) $m^3 \cdot s^{-1}$ (mean (SD)) during previous studies (Klotz 1985). The stream channel at this site was approximately 3 m wide and the bottom consisted primarily of cobblestones interspersed with coarse sediments, with some exposed bedrock upstream and downstream. The sediments at sites 4d and 4f had a much lower capacity for sorbing P than 4a sediments (Klotz 1988). Site 4f was located approximately 0.5 km upstream of a site with that designation used in a previous study (Klotz 1988); sediments and stream characteristics were similar for both locations.

Methods

Streamwater samples were collected at approximately monthly intervals from February to December 1988 at sites 4a and 4f. Collections were made in acid-cleaned, 1-L polyethylene bottles and stored on ice until returned to the laboratory. SRP was determined in duplicate on filtered (0.45 μm Millipore type HA, acid cleaned) streamwater samples the day of collection by the ascorbic acid method (APHA 1985) using a 10-cm cuvette. Ca was analyzed with a Perkin-Elmer model 303 atomic absorption spectrophotometer using alliquots of the same filtered samples. A composite sediment sample totalling 1 L was collected from the upper 7 cm of sediments at 10 locations at each site and either used fresh the day of collection for EPC_0 determination or dried (80°C) and stored for subsequent determination of sediment characteristics. The area of each site was great enough and the sampling interval was long enough so that collection did not significantly alter the sediments for future sampling.

EPC_0 was determined under standardized laboratory conditions by the technique described in Klotz (1988) which used a lower concentration of Ca in the incubating solution (to simulate streamwater conditions) than the original method (White and Beckett 1964; Taylor and Kunishi 1971). Duplicate sieved (2-mm mesh) sediment subsamples were incubated 1 h at 20°C in 50 mL of 20 mg $Ca \cdot L^{-1}$ ($CaCl_2$) solution spiked with KH_2PO_4 at initial P concentrations of 0, 10, and 25 $\mu g \cdot L^{-1}$. Flasks were shaken vigorously for 5 s every 10 min during the incubation. The aqueous and sediment phases were separated by filtration through acid-cleaned 0.45-μm membrane filters (Millipore type HA) and SRP was determined on the filtrate by the ascorbic acid method (APHA 1985). P desorption was calculated as the P released into the 0 $\mu g\ P \cdot L^{-1}$ solution during the EPC_0 determinations. Measured sediment subsamples were dried at 80°C to express EPC_0 and P desorption per unit dry weight. The effect of temperature on P desorption was determined on 4 August 1988 with site 4a sediments by conducting the above buffer experiments at 0 and 30°C in triplicate.

Several parameters considered potentially important in regulating EPC_0 over the long term were determined on dried sediments collected on each date of the annual analysis in 1988. Exchangeable Al and Fe were determined as described by Klotz (1988). Sediment P was measured by the technique of Sagher (1976). Organic matter content was estimated by weight loss

TABLE 1. Streamwater and sediment characteristics from monthly sampling in 1988.

Characteristic	Site 4a			Site 4f		
	Mean	SD	*n*	Mean	SD	*n*
SRP ($\mu g \cdot L^{-1}$)	2.73	1.08	13	6.72	2.86	13
EPC_0 ($\mu g \cdot L^{-1}$)	1.95	1.15	13	11.3	6.31	13
Streamwater Ca ($mg \cdot L^{-1}$)	19.1	5.6	11	21.8	5.4	11
Sediment P ($\mu g \cdot g^{-1}$)	223.0	22.1	13	42.6	13.3	13
Organic (%)	19.7	2.8	13	2.3	0.3	13
Exchangeable Fe ($\mu g \cdot g^{-1}$)	10.8	10.0	13	3.8	1.9	13
Exchangeable Al ($\mu g \cdot g^{-1}$)	33.1	5.9	13	3.8	2.7	13
Sediment ATP ($\times 10^{-9}$ $g \cdot g^{-1}$)	126	379	11	22	59	9
P desorbed ($\mu g \cdot g^{-1}$)	0.12	0.06	13	0.10	0.05	13

TABLE 2. Correlation coefficients for SRP and EPC_0 versus streamwater and sediment characteristics.

Characteristic	Site 4a		Site 4f	
	SRP	EPC_0	SRP	EPC_0
EPC_0	0.783[a]		0.603[b]	
Streamwater Ca	0.125	0.233	0.801[a]	0.677[b]
Sediment P	−0.131	0.179	−0.201	0.384
% organic	−0.732[a]	−0.631[b]	−0.227	−0.269
Exchangeable Fe	0.063	−0.117	0.141	0.240
Exchangeable Al	−0.275	−0.410	−0.114	−0.500
Sediment ATP	0.586	0.625[b]	0.453	0.252

[a] Significant at the 0.01 level.

[b] Significant at the 0.05 level.

of dried sediments at 500°C in a muffle furnace. Sediment ATP was extracted with DMSO (Palumbo et al. 1987); 1-mL fresh sediment samples were extracted in 20 mL of DMSO the day of collection for 24 h on a reciprocal shaker at 100 reciprocations per min.

Short-term changes in SRP were determined during a storm event on 29 July 1986 during which 1.1 cm of rain fell in a 15-min period. Streamwater samples and discharge measurements were taken prior to the storm, five times during the 2 h immediately following the storm and 5 and 22 h after the storm. Discharge was calculated using depth and velocity measurements at 11 points along a transect across the 1.3-m-wide stream 150 m upstream of site 4f, a location chosen because of its suitability for discharge measurements. Velocity determinations were made using a Gurley No. 625 Pygmy Current Meter (Teledyne Gurley, Troy, NY) at 0.6 depth. Water samples were collected at this location and analyzed for SRP and Ca as previously described. The influence of varying Ca on EPC_0 was determined on sediments collected on 11 September 1986 at the site of the storm event measurements. EPC_0 was determined as previously described except that the level of Ca in the incubating solution was adjusted to 18, 16, and 14 $mg \cdot L^{-1}$, simulating the streamwater Ca range measured during the storm event.

To test the effect of changes in streamwater Ca on SRP under controlled field conditions, the streamwater Ca concentration was experimentally increased in a section of stream 150 m upstream of site 4d. This location was chosen because the Ca rapidly mixed upon addition and a uniform channel with no pools allowed for unimpeded transport downstream. Two litres of $CaCl_2$ solution (72 g $Ca \cdot L^{-1}$) was metered into the stream over a period of 7 min 40 s on 27 July 1987. Fifteen streamwater samples were collected 7.6 m downstream over a 22-min period from the start of the spike and analyzed for Ca and SRP as previously described. The mixing of the $CaCl_2$ solution and the progression of the spike downstream were monitored by conductivity measurements. The Ca change chosen for use during the spike was greater than the change measured during the storm event because of experimental necessity; the experimental change needed to be great enough to produce measureable SRP changes just 7.6 m downstream. It would be expected that a change in Ca during a storm event would produce relatively greater changes in SRP due to the additive effect of the stream water contacting sediments during a longer time over the entire stream length.

Results

Although the two sites studied were distinctly different (Table 1), streamwater SRP was significantly correlated with sediment EPC_0 at both sites 4a ($r = 0.783$; $p < 0.01$; $n = 13$) and 4f ($r = 0.603$; $p < 0.05$; $n = 13$) over an annual cycle (Table 2; Fig. 2). Over the 11-mo period of study in 1988, site 4a had distinctly lower SRP and EPC_0 and higher sediment P, organic content, exchangeable Al and Fe, and sediment ATP than site 4f (Table 1). For site 4a, SRP and EPC_0 were negatively correlated with percent organic matter and EPC_0 was positively correlated with sediment ATP (Table 2), indicating that P release during the decomposition of organic matter may have been an important source of SRP. Further support for this explanation was provided by laboratory experiments which showed that P desorption from site 4a sediments was significantly greater ($p < 0.01$) when incubations were carried out at 30 versus 0°C (Table 3). For site 4f, SRP and EPC_0 were positively correlated with streamwater Ca but not with organic content or sediment ATP (Table 2). Also at site 4f, P desorption into 0 μg $P \cdot L^{-1}$ solution during the EPC_0 determinations was positively correlated ($r = 0.651$; $p < 0.05$) with streamwater Ca. These results for site 4f indicated that geochemical processes in the sediments as influenced by streamwater Ca may have been important in determining long-term streamwater SRP variations. Streamwater Ca increased during the low-discharge months of summer and early fall at both sites 4a and 4f (Fig. 3).

The short-term relationship between streamwater Ca and SRP was determined near site 4f following a 1.1-cm storm event between 1540 and 1555 on 29 July 1986. SRP increased and streamwater Ca decreased during the event, the maximum SRP and the minimum Ca occurring on the descending limb of the runoff hydrograph (Fig. 4). Ca decreased from 17.8 $mg \cdot L^{-1}$ before the start of the storm event to 14.1 $mg \cdot L^{-1}$ on the descending limb of the runoff hydrograph, while SRP increased from 14.3 to 20.1 $\mu g \cdot L^{-1}$ (Fig. 4). SRP and Ca were significantly negatively correlated ($r = -0.791$; $p < 0.05$). Streamwater pH did not show significant changes during the storm event, remaining in the 7.7 to 7.8 range, and therefore did not influence SRP changes by influencing the phosphate buffer mechanism.

Other factors besides streamwater Ca may have changed during the storm event to result in elevated streamwater SRP. To determine how Ca influenced SRP under controlled laboratory conditions, the changes in streamwater Ca experienced during the storm event were mimicked by sorption experiments in which dissolved Ca was adjusted to 18, 16, and 14 $mg \cdot L^{-1}$. The decrease in Ca in the incubating solution of the buffer experiments led to an EPC_0 increase from 12.3 to 15.4 $\mu g \cdot L^{-1}$

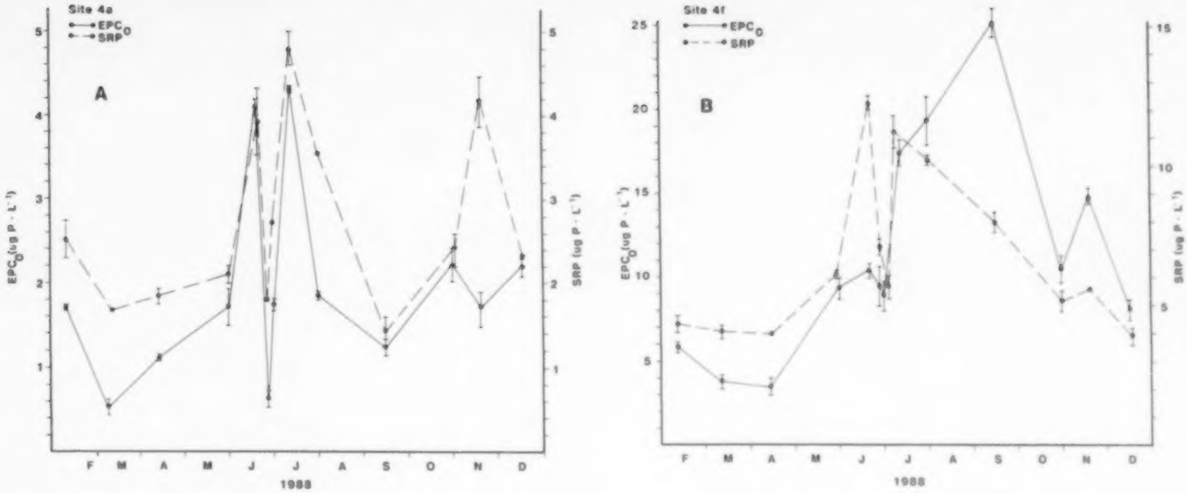

FIG. 2. Zero equilibrium phosphate concentration (EPC$_0$) and soluble reactive phosphorus (SRP) at (A) site 4a and (B) site 4f during 1988. Each point is the mean of duplicate samples; vertical bars are SD.

TABLE 3. Soluble reactive phosphorus (SRP) released from site 4a sediments at two temperatures. Values are means (SD) of triplicate analyses; units are μg P·mL sediments^{-1} ($\times 10^{-3}$) released during 1 h of incubation.

Incubation temperature (°C)	SRP released[a]
0	8.0 (0.8)
30	22.2 (1.6)

[a] Values for the two temperatures significantly different at the $p<0.01$ level.

TABLE 4. Effect of dissolved Ca on EPC$_0$ of sediments collected near site 4f on 11 September 1986.

	EPC$_0$ (μg·L^{-1})		
Ca (mg·L^{-1})	Mean	SD	*n*
18	12.3	0.16	2
16	13.7	0.19	2
14	15.4	0.41	2

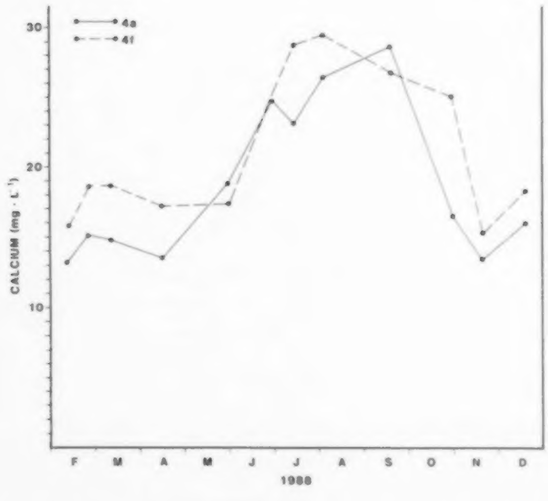

FIG. 3. Streamwater Ca at sites 4a and 4f during 1988. Each point is the mean; SD values were too small to be shown on this scale.

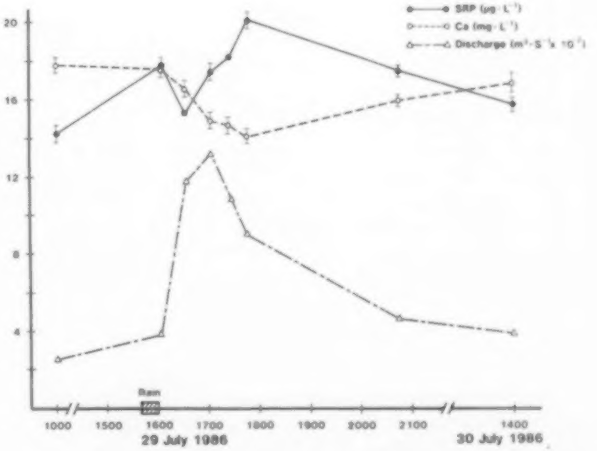

FIG. 4. Streamwater soluble reactive phosphorus (SRP) and Ca discharge at a site 150 m upstream of site 4f during a storm event on 29–30 July 1986. For SRP and Ca, each point is the mean; vertical bars are SD.

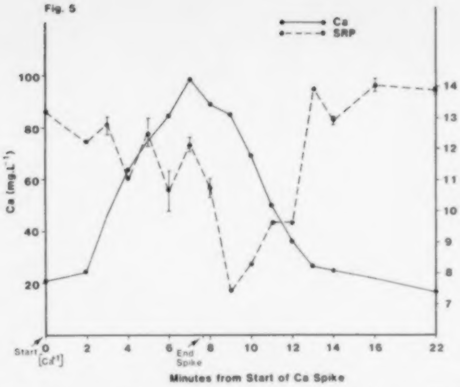

FIG. 5. Streamwater Ca and soluble reactive phosphorus (SRP) during a $CaCl_2$ spike of the stream 150 m upstream of site 4d on 27 July 1987.

(Table 4). Under conditions of comparable decreases in dissolved Ca, the laboratory EPC_0 increase of 3.1 $\mu g \cdot L^{-1}$ was 53% of the 5.8 $\mu g \cdot L^{-1}$ increase in streamwater SRP during the storm event. This indicates that decreased streamwater Ca during the storm event was probably partially responsible for elevated SRP by influencing the phosphate buffer mechanism.

The ability of short-term changes in streamwater Ca to influence SRP was also tested experimentally in the stream near site 4d. When streamwater Ca was experimentally increased by spiking with $CaCl_2$, SRP decreased (Fig. 5). The correlation coefficients were -0.574 ($p < 0.05$) when Ca and SRP from the same sampling times were evaluated, -0.714 ($p < 0.01$) when Ca was evaluated with SRP from one sampling time later, -0.794 ($p < 0.01$) when Ca was evaluated with SRP from two sampling times later, and -0.675 ($p < 0.05$) when Ca was evaluated with SRP from three sampling times later. The fact that the most significant correlation occurred when Ca was evaluated with SRP from two sampling times later indicated that it may have taken a brief time (approximately 2 min) for Ca to fully influence streamwater SRP, a delay probably due to the time required for diffusion to occur between stream water and the interstitial water of the sediments.

Discussion

Long-term Changes in SRP and EPC_0

A previous study on Hoxie Gorge Creek showed that EPC_0 was positively correlated with SRP spatially at sites 4a to 4f (Klotz 1988); the present study found that EPC_0 and SRP were also positively correlated temporally over an annual cycle (Table 2). Over this extended time period, SRP and EPC_0 were consistently lower at site 4a than site 4f (Table 1; Fig. 2), a finding which confirms that the differences noted previously (Klotz 1988) in P regulation between the sediments at these two sites exist throughout the year. The fact that the positive correlation between EPC_0 and SRP existed over the annual cycle for these two diverse sites with distinctly different sediment types (Table 1) lends credence to the possibility that it may be a widespread phenomenon. To this author's knowledge, no other study has considered the association between streambed sediment EPC_0 and SRP over an annual cycle.

The correlation coefficient for EPC_0 versus SRP at site 4f would probably have improved if the exact Ca concentration of the stream water had been used in the incubating solutions for the EPC_0 determinations on each date. It was decided for reasons of standardization to use the same Ca concentration (20 $mg \cdot L^{-1}$) in the incubating solution on all dates. However, previous work showed that dissolved Ca in the incubating solution was negatively related to EPC_0, a mechanism that produced larger changes in EPC_0 relative to Ca at site 4f than at site 4a (Klotz 1988). Thus, the EPC_0 values reported here underestimated the true EPC_0 in the stream when streamwater Ca was below 20 $mg \cdot L^{-1}$ and overestimated when streamwater Ca was above 20 $mg \cdot L^{-1}$. Ca at site 4f was below 20 $mg \cdot L^{-1}$ through the 1 June 1988 sample (Fig. 3), and therefore, EPC_0 measurements underestimated true stream EPC_0. On these dates at site 4f, EPC_0 values would have been higher and probably closer to SRP (Fig. 2). Using the same reasoning, the August through October EPC_0 values likely overestimated true streamwater EPC_0; EPC_0 values would have been lower and probably closer to SRP (Fig. 2) if exact streamwater Ca concentrations were used in the incubating solution.

The long-term changes in EPC_0 at sites 4a and 4f (Fig. 2) were as great as the spatial change in EPC_0 between the two sites determined during this study. Spatial change, as shown by the daily difference in EPC_0 between sites 4a and 4f during 1988, had a range of 21.6 $\mu g\ P \cdot L^{-1}$, while the seasonal range in EPC_0 was 3.86 $\mu g\ P \cdot L^{-1}$ at site 4a and 21.6 $\mu g\ P \cdot L^{-1}$ at site 4f. The seasonal range at site 4a was less than that at site 4f probably because EPC_0 was lower at site 4a and its sediments have a greater capacity to sorb large quantities of P (Klotz 1988), thus providing a greater buffering capacity for streamwater SRP.

The factors regulating the long-term changes in EPC_0 appear to be different from those which were found previously to regulate spatial changes in Hoxie Gorge Creek (Klotz 1988). Spatial changes in EPC_0 were associated with a decrease in the levels of exchangeable Al as streambed sediments became more coarse from site 4a to 4f (Klotz 1988). In the present study, neither exchangeable Al nor exchangeable Fe was correlated with EPC_0 (Table 2), indicating that they were probably not important in regulating annual EPC_0 changes.

The reasons for annual EPC_0 change appear to be different for sites 4a versus 4f, a conclusion reached because of the higher organic content and ATP (indicative of biological activity) at site 4a (Table 1) and the differences in correlations with sediment characteristics at the two sites (Table 2). Although correlations do not prove cause and effect, the significant negative correlation between EPC_0 and percent organic matter and the positive correlation between EPC_0 and sediment ATP (Table 2) indicate that P regeneration due to biological decomposition of organic matter may have been a factor responsible for EPC_0 increases at certain times at site 4a. P regeneration by mineralization of organic phosphorus compounds is important in the aerobic sediments of some lakes (Lee et al. 1977; Boers and van Hese 1988; Sinke and Cappenberg 1988). The highest EPC_0 value at site 4a (4.32 $\mu g \cdot L^{-1}$) occurred on 11 July 1988, the same date as the highest sediment ATP was measured (1.27×10^{-6} g ATP·g dry wt. $sed.^{-1}$), indicating high biological activity. The maximum daily temperatures for the week prior to this ranged from 31.7 to 33.9°C with no rain. Discharge was very low. Also, the date (20 June 1988) with the next highest EPC_0 at site 4a (4.17 $\mu g \cdot L^{-1}$ corresponded with another very low discharge period, with only a total of 0.1 cm of rain for the 17 d prior to this date. ATP was not measured on this date. A sharp decrease in SRP and EPC_0 occurred at site 4a on 30 June 1988, coinciding with much cooler temperatures (maximum daily temperature of 11.7°C). P release from site 4a sediments was greater at 30 versus 0°C in laboratory experiments (Table 3), a result expected if biological activity was responsible for the increase. It appears that low discharge and high temperatures at site 4a may have led to increased biological activity and P regeneration through microbial decomposition.

The phosphate buffer mechanism may be responsible for the change in EPC_0 over the annual cycle at site 4f, a site lower in organic content and ATP than site 4a. EPC_0 was positively correlated with Ca over the long term at site 4f (Table 2), a result opposite that found for short-term EPC_0 versus Ca relationships in laboratory buffer experiments and during storm events (Klotz 1988) (Table 4; Fig. 4). Despite this seeming incongruity, both behaviors might be explained by the same basic mechanism. This possible explanation is that high streamwater Ca during low-flow summer months (Fig. 3) led to increased P sorption by the sediments by the phosphate buffer mechanism described in Klotz (1988). Increased P sorption by the sediments can lead to increased EPC_0 (Froelich 1988). A reason that this mechanism would be more important at site 4f is because EPC_0 changes were more sensitive to dissolved Ca variation at site 4f than at site 4a (Klotz 1988). Others have also shown that during low summer flows, P is retained in the streambed (Rigler 1979; Hill 1982), but they did not relate the behavior to the P sorption mechanism. In support of the above explanation is the positive correlation ($p < 0.05$) between P desorption and streamwater Ca at site 4f. Others have used P desorption, or the amount of P released into $CaCl_2$ solution, as a measure of soluble P in sediments (Sah and Mikkelsen 1986).

Other factors may also be responsible for higher EPC_0 and SRP at site 4f during the low-discharge summer months. It is possible that increased streamwater SRP led to higher EPC_0 in the sediment due to greater P sorption (Froelich 1988). The increased streamwater SRP may have resulted from greater input of SRP from upstream sites by the processes discussed above for site 4a. The SRP concentration may also have increased during the low-discharge summer months due to less dilution or due to changes in the relative contributions of various streamwater sources (Rigler 1979).

Short-term Changes in SRP and EPC_0

Both laboratory and field data have made a strong case for the importance of ionic strength (as influenced by Ca) in regulating short-term changes in streamwater SRP by means of the phosphate buffer mechanism. Evidence supporting the inverse relationship between streamwater Ca and SRP came from analyses of Ca and SRP changes during a storm event (Fig. 4), controlled stream experiments (Fig. 5), and laboratory buffer experiments (Table 4). The fact that the ionic strength of the aqueous solution influences P sorption is well known by soil scientists, but only recently was it shown to be important in streams (Klotz 1988). Ca is the dominant cation in Hoxie Gorge Creek (Klotz 1985) and it is particularly effective in influencing P sorption because of its divalent charge (Klotz 1988). For these reasons it is satisfactory for this study to consider only Ca when discussing factors related to ionic strength.

The fact that the laboratory EPC_0 change (Table 4) accounted for only 53% of the streamwater SRP change (Fig. 4) under conditions of comparable decreases in dissolved Ca indicated that other factors probably act in concert with ionic strength and cation changes to cause SRP changes during storm events. Taylor and Kunishi (1971) found that EPC_0 was higher in upper soil layers; during a storm event these upper layers may contribute more water to stream flow and thus increase streamwater SRP. Rewetting of dried sediments during a storm event would be expected to increase streamwater SRP because sediments that have been dried have higher EPC_0 values than if they had remained moist (Twinch 1987; Klotz 1988). Rainfall with higher P than stream baseflow concentrations was also suggested as a reason for elevated streamwater P during storm events (Lowrance and Leonard 1988). Rigler (1979) concluded that soluble P accumulates on the streambed during baseflow and predominates during the late descending limb of the runoff hydrograph. He explained streamwater P behavior during storm events in terms of variation in P input via shallow and deep throughflow at different times during the storm event.

Some, but not all, streams studied by others showed elevated SRP during storm events. Georgia coastal plain streams exhibited elevated ortho-P levels and decreased conductivity during storm events (Lowrance and Leonard 1988). The decreased conductivity probably indicated decreased ionic strength and cation concentration, and therefore, part of the P increase may have occurred as a result of the phosphate buffer mechanism. A first-order Pennsylvania stream showed decreased Ca and increased SRP during the stages of increasing stream discharge (McDiffett et al. 1989). SRP did not change during storm events in Bear Brook (Meyer and Likens 1979), perhaps because streamwater Ca was low initially ($1.65\ mg \cdot L^{-1}$; Likens et al. 1977), and therefore the magnitude and range of the ionic strength change would not significantly influence the phosphate buffer mechanism.

The analysis of nutrient transport relative to runoff hydrographs has been used as a means of assessing the sources (e.g. groundwater, overland flow, interflow, terrestrial, and streambed sources) of the various nutrients (Bond 1979; Rigler 1979; Meyer and Tate 1983). Data reported here indicate that the interpretation of SRP behavior in relation to the runoff hydrograph should be done with knowledge of sorption processes, and should consider the influence of streamwater ionic strength and cations on the phosphate buffer mechanism. SRP changes at certain stages of the hydrograph may simply reflect changes in sediment sorption equilibria due to changes in streamwater cations such as Ca, and not changes in the source of the P. This phenomenon may not be limited to P, for other elements are bound to sediments in a similar way as P (Froelich 1988) and therefore would be expected to respond to changes in streamwater ionic strength.

Acknowledgements

I thank D. J. Birdsey, J. Dykstra, A. Funk, B. Hauber, C. O'Brien, B. Over, C. Richardson, and P. Ventiquattro for assistance with various aspects of this study. J. Bugh unselfishly compiled the weather records. This research was supported by a grant to the author from the National Science Foundation (BSR-8500225).

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Transport, Distribution, and Abundance of Larval and Juvenile Walleye Pollock (*Theragra chalcogramma*) in the Western Gulf of Alaska

Sarah Hinckley, Kevin M. Bailey, and Susan J. Picquelle

Alaska Fisheries Science Center, National Marine Fisheries Service, 7600 Sand Point Way NE, Seattle, WA 98115, USA

James D. Schumacher and Phyllis J. Stabeno

Pacific Marine Environmental Laboratory, National Oceanic and Atmospheric Administration, 7600 Sand Point Way NE, Seattle, WA 98115, USA

Hinckley, S., K. M. Bailey, S. J. Picquelle, J. D. Schumacher, and P. J. Stabeno. 1991. Transport, distribution, and abundance of larval and juvenile walleye pollock (*Theragra chalcogramma*) in the western Gulf of Alaska. Can. J. Fish. Aquat. Sci. 48: 91–98.

The spawning distribution of walleye pollock (*Theragra chalcogramma*) and subsequent southwesterly drift of eggs, larvae, and juveniles were investigated in Shelikof Strait, March–September 1987. In mid-March, a hydroacoustics survey found that spawning adults were concentrated in the deeper parts of Shelikof Strait, as were eggs collected in this area during an ichthyoplankton survey about 1 mo later. In May, a concentration of young larvae was found 100–150 km to the southwest of the spawning area. In late June and early July, the center of distribution of late larval and early juvenile walleye pollock was further to the southwest, between the Shumagin and Semidi Islands. By August and September, juveniles were mostly distributed downstream of the Shumagin Islands. The rate of drift of eggs and larvae through June was estimated at 4–6 cm/s. The trajectories of satellite-tracked buoys deployed in the region of greatest egg abundance revealed similar patterns to those of the early life stages of walleye pollock. These observations demonstrate that transport is an important factor determining the distribution of pollock larvae in downstream coastal nursery areas.

On a étudié la distribution du frai de la morue du Pacifique occidental (*Theragra chalcogramma*), et la dérive subséquente des oeufs, des larves et des juvéniles en direction sud-ouest dans le détroit Shelikof, de mars à septembre 1987. À la mi-mars, une étude hydroacoustique a montré que les adultes qui frayent étaient concentrés dans les parties plus profondes du détroit Shelikof, tout comme les oeufs cueillis dans cette zone au cours d'une étude de l'ichtyoplancton réalisée environ un mois plus tard. En mai, on a trouvé une concentration de jeunes larves à une distance de 100–150 km au sud-est de la zone de frai. Fin juin et début juillet, le centre de distribution des larves avancées et des juvéniles précoces de la morue du Pacifique occidental était situé plus au sud-ouest, entre les îles Shumagin et Semidi. En août et en septembre, les juvéniles étaient surtout distribués en aval des îles Shumagin. Le taux de dérive des oeufs et des larves au cours du mois de juin a été évalué à 4–6 cm/s. Les trajectoires des bouées poursuivies par satellite installées dans la zone où les oeufs étaient plus abondants ont révélé des distributions semblables à celles qui sont observées au cours des stades précoces de la morue du Pacifique occidental. Ces observations montrent que le transport est un facteur important dans la distribution des larves de morue du Pacifique occidental dans les régions d'alevinage côtières situées en aval.

Received September 26, 1989
Accepted July 20, 1990
(JA320)

Reçu le 26 septembre 1989
Accepté le 20 juillet 1990

In the western Gulf of Alaska, walleye pollock (*Theragra chalcogramma*) spawn primarily in late March and April in Shelikof Strait, a narrow sea valley located between Kodiak Island and the Alaska Peninsula (Kim 1987; Kendall et al. 1987) (Fig. 1). Although walleye pollock spawn in other parts of the western Gulf of Alaska, the Shelikof Strait spawning area is thought to be the most important. Spawning occurs in the deepest part of the sea valley, with eggs mostly found below 150 m (Kim 1987). Eggs hatch in mid-April to mid-May after about a 2-wk incubation period (at approximately 5°C, Incze et al. 1989). Larvae are 3–4 mm standard length (SL) at hatching. Larvae hatch at depth and due to positive buoyancy rise to the upper 50 m of the water column by late May (Kendall et al. 1987). Eggs and larvae are often found in distinct patches (Incze et al. 1989, 1990). Very low egg concentrations have been found outside Shelikof Strait in most years (Kendall and Picquelle 1990).

Current velocities at the depth of maximum egg abundance are weak, and eggs probably do not drift far from the area where they are spawned (Kim 1987). Larvae are transported by the currents in the upper 50 m of the water column (Incze et al. 1989). Larval drift to the southwest in April and May is well documented (Kendall et al. 1987; Kim 1987).

The nursery area for juvenile walleye pollock resulting from the Shelikof Strait spawning has been assumed to be over the continental shelf somewhere along the Alaska Peninsula. Prior to this study, however, little evidence for the coastal nursery area had been presented. Juvenile groundfish surveys in the northwestern Gulf of Alaska conducted in the late summer and early fall from 1980 to 1982 (Smith et al. 1984) and from 1984 to 1988 (Alaska Fisheries Science Center (AFSC), Seattle, WA

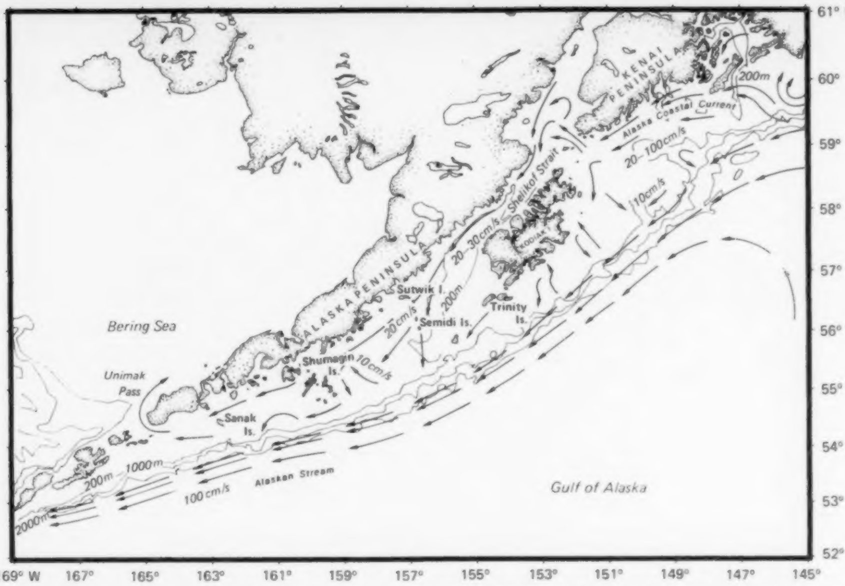

FIG. 1. Major water circulation features in the western Gulf of Alaska (from Schumacher and Reed 1986a).

98115, unpubl. data) concentrated mainly on the bays around Kodiak Island and several bays and nearshore areas along the Alaska Peninsula. These surveys covered little of the offshore shelf areas and may not have included the major nursery grounds for Shelikof Strait walleye pollock. Little information has been available on the distribution, abundance, and biology of late larval and early juvenile stages (metamorphosis occurs at about 30 mm, J. R. Dunn, AFSC, Seattle, WA, pers. comm.) of walleye pollock between late May and mid-August.

In this study we track the distribution of spawning adults, eggs, and young larvae in 1987. These data are compared with the subsequent distribution of late larvae and juveniles. The results of an exploratory survey conducted in June and July of 1987 to assess the geographical distribution and abundance of the late larval and early juvenile stages of walleye pollock are described in detail. Movements of satellite-tracked buoys (drogued at 40 m and released in the main area of distribution of eggs and larvae in April and May) are presented and compared with the drift of larvae through July. Finally, we examine the feasibility of using a survey during the June–July time period for estimation of year-class strength.

Materials and Methods

A hydroacoustic midwater trawl survey for the assessment of spawning adult walleye pollock biomass and biological characteristics was conducted in Shelikof Strait between March 11 and 17, 1987 (Nunnallee 1987). Acoustic data were collected using a computerized echo-integration and dual beam target strength measurement system. Species composition, age, length, and maturity data were collected from midwater trawl samples.

Within Shelikof Strait, a survey for eggs was conducted April 2–17; outside the strait, the survey period for eggs was April 9–27. The young larval survey was conducted May 18–29. Sampling gear for the egg and early larval surveys consisted of 20- and 60-cm bongo nets (150- and 505-μm mesh, respectively, on the egg survey; 150- and 333-μm on the young larval survey). Methods for our egg and young larval surveys are described in detail in Dunn and Rugen.[1]

Methods for the June–July survey for late larvae and early juveniles are given in more detail because of its possible use in prerecruit surveys. The survey area (Fig. 2) for the June–July cruise covered most of the western Gulf of Alaska. Transect lines were placed 50 km apart, crossing the continental shelf perpendicular to the Alaska Peninsula. Stations were 25 km apart along each transect line. Transect lines extended from nearshore to beyond the 200-m depth contour. The survey began at the southwest end of the grid on June 18 and proceeded northeastward, finishing on the eastern side of Kodiak Island on July 16; 148 stations were occupied.

The primary sampling gear for the June–July survey was the Methot frame trawl (Methot 1986). The Methot trawl is a micronekton trawl designed to catch larval and juvenile fishes. The net has a 5-m^2 rigid frame and is equipped with 2 × 3 mm oval mesh and a hard codend with 505-μm mesh. The net was towed in an oblique or stepped oblique manner from a maximum depth of 300 m to the surface. Retrieval speed was 20 m/min (ship speed was about 2–2.5 knots). A flowmeter was used to estimate the volume of water filtered. Net depth was estimated using a mechanical bathykymograph.

Numbers of larvae captured were standardized into numbers per 10 m^2 surface area using the volume filtered and depth (Smith and Richardson 1977). A nonparametric analysis of variance (ANOVA), the Kruskal–Wallis H statistic (Zar 1984), was used to analyze day and night differences in the catch due to heteroscedasticity of the data. Strata (see below) were included as a factor in the ANOVA to take into account the strong regional effect on larval abundance and to correct for

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[1] Dunn, J. R., and W. C. Rugen. 1989. A catalog of Northwest and Alaska Fisheries Center ichthyoplankton cruises 1965–1988. NWAFC Processed Rep. 89-04. Alaska Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, 7600 Sand Point Way NE, Seattle, WA 98115, USA.

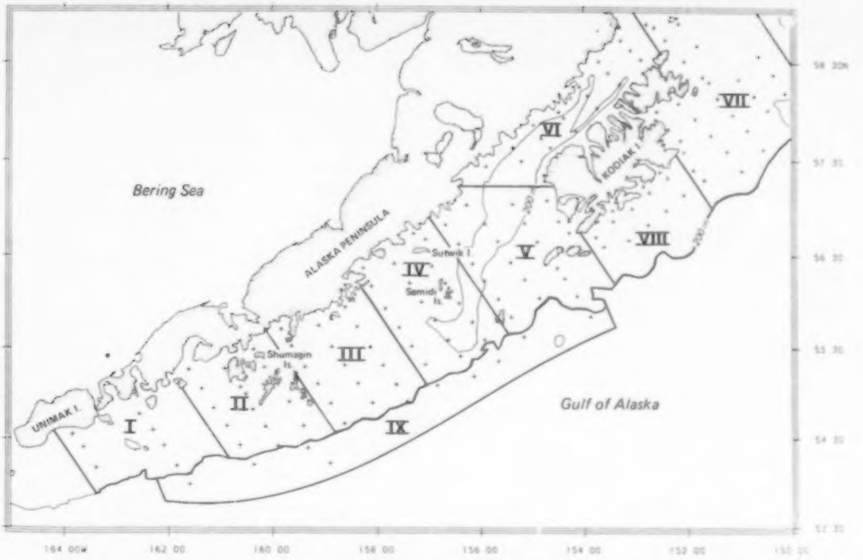

FIG. 2. Survey area in the western Gulf of Alaska included in the 1987 cruise for late larval and early juvenile walleye pollock (+ = station location). The nine strata used to calculate abundance are also shown.

any uneven sampling among strata over the 24-h cycle. Only strata with more than 50% positive stations were considered in order to exclude areas outside the range of young pollock from the test.

The total abundance of walleye pollock in June and July was estimated by dividing the survey grid into nine strata of roughly equal size (Fig. 2). A stratified sampling scheme was used to reduce the variance of the overall abundance estimate and to more efficiently allocate sampling effort (putting fewer stations in areas where few larvae are expected) (Jessen 1978). Because we expected an along-shelf gradient of abundance, eight of these strata were over the continental shelf. One additional stratum included all stations located in waters greater than 200 m deep where we expected fewer larvae (Fig. 2). Mean abundances and variances were calculated for each stratum. Total abundance was the sum of the stratum means, weighted by the area of each stratum. The variance of the total estimate was the sum of the stratum variances (weighted by the squared stratum areas).

Eight satellite-tracked buoys were deployed in a line across lower Shelikof Strait on April 12, 1987. Approximately a month later, another 10 were deployed along the same line. Each buoy was equipped with a "holey sock" drogue (a fabric cylinder with holes, which is oriented vertically in the water) centered at 40 m, the assumed depth of the highest larval abundance (Kendall et al. 1987). On average, eight reports of positions were received from the system Argos satellite each day. Fifteen of the 18 buoys returned useful data.

The survey for late juveniles in 1987 took place from August 12 to September 20. This survey made transects from Unimak Pass to the eastern side of Kodiak Island, trawling with a 24-m high-opening shrimp trawl on targets observed on the ship's echo sounder.

Results

Distribution of Adults, Eggs, and Young Larvae

Prespawning adult walleye pollock migrated into Shelikof Strait from the southwest in February and early March. By mid- to late March, the highest concentrations were found over the deepest waters of the strait near Cape Kekurnoi (Fig. 3).

The highest numbers of eggs were found in early April near the location of maximum spawner abundance (Fig. 4), and there were very low egg concentrations outside the strait. The survey for young larvae conducted in May only covered a part of the distribution resulting from the Shelikof Strait spawning. Relatively high abundances were found, however, in a patch east of Sutwik Island and the Semidi Islands, approximately 130 km downstream of the area of highest egg abundance (Fig. 5).

Distribution of Late Larvae and Juveniles

Highest abundances of late larvae and early juveniles were found between the Semidi Islands and the Shumagin Islands (Fig. 6). Catches in these areas ranged from 30 to 85 fish/10m^2. The variation in catch between adjacent stations was low at this scale of sampling. Numbers of late larvae and early juveniles were concentrated over the continental shelf, with 92.5% found in strata II, III, and IV. Only 1% of all larvae found were in strata I, and even less were collected off the continental shelf (0.5%, strata IX). Very few late larvae or early juvenile walleye pollock were found around Kodiak Island (6.0% in strata V–VIII).

Most of the older juveniles caught during the August–September cruise were found among and to the west of the Shumagin Islands (Fig. 7). Although capturing the main features of the overall distribution, contouring obscured the high station-to-station variability observed at this life stage. Distributions of juveniles were patchy (with numbers at nearby stations varying from 0 to greater than 1000 fish/10-min tow).

Water Movement and Properties

Between April and July, four satellite-tracked buoys drifted to the southwest out of Shelikof Strait and travelled over the continental shelf, close to the Alaska Peninsula (Fig. 8). Seven

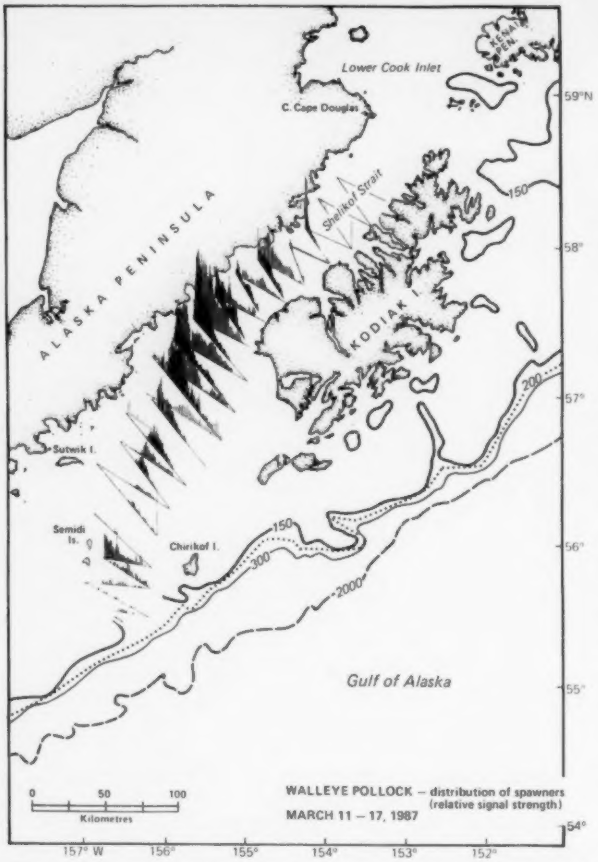

FIG. 3. Relative signal strength of walleye pollock in Shelikof Strait March 11–17, 1987, as determind by the hydroacoustics survey (Nunnallee 1987).

buoys moved seaward through the Shelikof sea valley, passed between the Semidi Islands and Chirikof Island, and returned to the continental shelf area rather than becoming entrained in the Alaskan Stream off of the shelf. The remaining four buoys moved along the sea valley, out into the Alaskan Stream, and were transported rapidly to the west, seaward of the continental shelf and beyond the coastal nursery area.

At the time of the young larval survey (May 18–29), four of the six still-functional buoys from those released in April were located in the area just west of Sutwik Island and the Semidi Islands. Over this period, movement of the buoys indicated many perturbations in the current and weak mean flow (<3cm/s) and none moved more than about 30 km in any one direction.

Of the 11 buoys which continued to transmit after May, seven were in the region of the Shumagin Islands in June. This is also the location of the highest concentration of late larvae and early juveniles (Fig. 6).

By approximating the center of distribution of walleye pollock eggs and larvae, and measuring its displacement between cruises, we estimated a rate of drift approximately 4–6 cm/s between the time of the egg survey and the late larval and early juvenile survey. Between the egg survey and the young larval survey, the approximate rate of drift was 3–4 cm/s; between the young larval and the late larval survey, the rate of drift was about 5–6 cm/s.

Abundance Estimation

Because the day and night effect was not significant ($\chi^2 = 1.36, p = 0.71$; Table 1), no adjustment was made to the abundance estimates to correct for differential catchability. The strata effect was significant ($p = 1.0$), which supports using the stratified mean to estimate total abundance. The stratified estimate resulted in a total abundance of late larvae and early juveniles of 9.02×10^{10} (SE = 1.34×10^{10}).

Discussion

Near-surface water circulation in Shelikof Strait is dominated by the Alaska Coastal Current (ACC) (Fig. 1), a narrow (20 km wide), high-transport (about 0.85×10^6 m^3/s) feature which flows southwestward (Reed et al. 1989; Schumacher et al. 1989). Current speeds vary seasonally and can exceed 30 cm/s in the spring (fig. 7 in Incze et al. 1989). The ACC divides near the Semidi Islands (Fig. 1), with 25% of the transport flowing westward along the Alaska Peninsula and the remainder flowing along the sea valley into the Gulf of Alaska (Schumacher and Reed 1986b; Schumacher et al. 1989). Water exiting the Shelikof sea valley may eventually return to coastal areas (Reed et al. 1987). Less is known about the currents over the continental shelf to the west of the Semidi Islands, but they are generally weaker, with speeds of 5–15 cm/s.

Hydroacoustics surveys since 1980 have found most adult walleye pollock spawning near Cape Kekurnoi (Fig. 3), over a deep (250–300 m) trench. The restricted spawning area, together with eddies (Incze et al. 1989, 1990) and other physical factors which result in convergence (Reed et al. 1989), may contribute to the aggregation or patchiness of eggs and young larvae. In 1987, a patch of young larvae was found near Sutwik and the Semidi Islands. Concentrations were also found in this area in 1981 and 1985 (Incze et al. 1989).

The June–July 1987 survey showed that late larval and early juvenile stages of walleye pollock are found in coastal nursery areas over the continental shelf along the Alaska Peninsula, with maximum concentrations between the Semidi and Shumagin Islands. Few fish were found in the bays around Kodiak Island during this survey or during the August–September survey for older juveniles. In some years (especially 1985 and 1988), the late summer and early fall surveys found older juveniles in the bays and waters around Kodiak Island in addition to concentrations along the Alaska Peninsula. Juveniles found in other years in these areas may have derived from spawning which may occur to the north and east of Kodiak. The existence of significant spawning concentrations in these areas has, however, never been documented. A hydroacoustic survey of the western Gulf of Alaska in 1990, for example, found little evidence of spawning in other areas (J. Traynor, AFSC, Seattle, WA, pers. comm.). Anomalous transport of eggs and larvae and migration of juveniles may be other alternatives.

The location of the satellite-tracked buoys in mid- to late May was somewhat west of the observed distribution of young larvae. One possible explanation for this is that the actual larval distribution extended west of the limited area surveyed during the cruise. Alternatively, since the buoys were deployed at the time of assumed highest egg abundance (or perhaps 1 wk earlier, according to Yoklavich and Bailey 1990) and they were

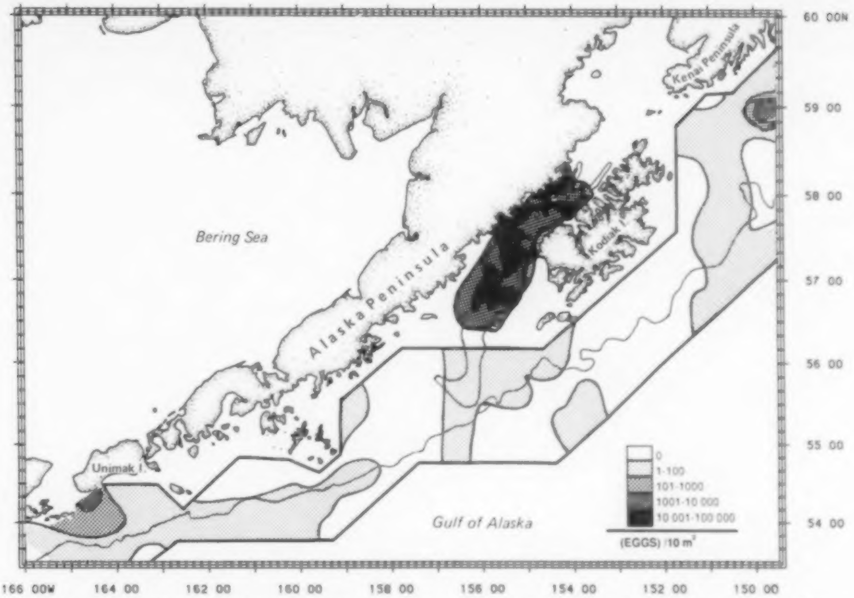

FIG. 4. Distribution of walleye pollock eggs in April 1987. Solid lines indicate survey boundary.

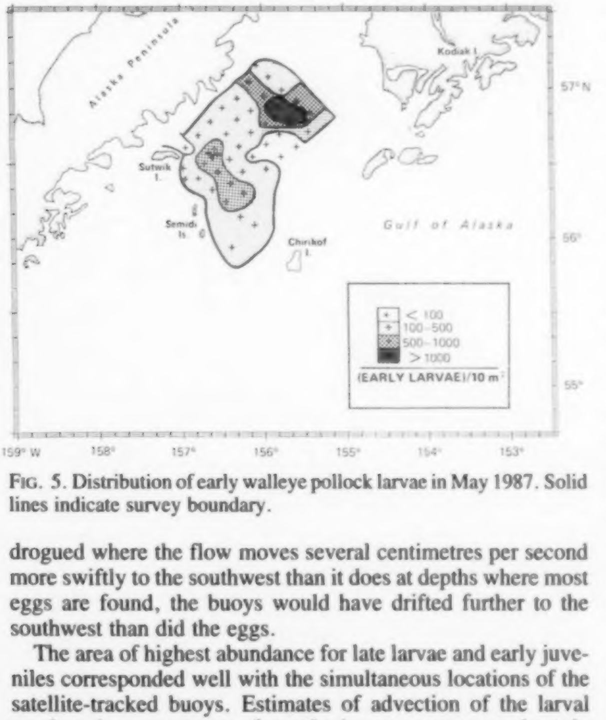

FIG. 5. Distribution of early walleye pollock larvae in May 1987. Solid lines indicate survey boundary.

drogued where the flow moves several centimetres per second more swiftly to the southwest than it does at depths where most eggs are found, the buoys would have drifted further to the southwest than did the eggs.

The area of highest abundance for late larvae and early juveniles corresponded well with the simultaneous locations of the satellite-tracked buoys. Estimates of advection of the larval patch and measurements from the buoys were approximately 4–6 cm/s. Yoklavich and Bailey (1990) noted that the birthdate distribution of larvae caught during the May survey (corrected for size-selective mortality) indicates that these larvae derive from eggs spawned 1–2 wk after the April egg survey. Rates of drift using their estimate of mean spawning date of April 20 would be 5–7 cm/s (assuming that the later spawning occurred in the same location as that seen during the earlier survey). Kim (1987) found similar rates of drift for eggs and larvae (April and May of 1981 and 1983) of 2–8 cm/s. Current speeds in the ACC, the locally dominant current system, are 5–30 cm/s in April–June, but shoreward of this feature and over the continental shelf between the Semidi and the Shumagin Islands, flow is weak and can sometimes reverse. The relatively slow rates of larval drift compared with potential transport rates of the ACC may indicate that larvae were not hatched into the ACC, but remained in the weaker, variable current regime west and shoreward of the ACC, or in eddies that act as retention mechanisms (Incze et al. 1989). The region from Sutwik and the Semidi Islands to the Shumagin Islands may therefore serve as a nursery area for larvae and early juveniles. It is possible that later juveniles are located here as well; however, this area was not well covered during the August–September survey.

Using the buoy data from 1987 and moored current meter data (Schumacher et al. 1989), we estimate that approximately 75% of the upper (less than about 60 m) water transport through Shelikof Strait flows along the Alaska Peninsula, and 25% exits the shelf to join the Alaskan Stream. Annual variation in these percentages could affect the proportion of larvae carried to offshore areas, where their chances for survival could be lessened. For example, in 1985, increased offshore transport was hypothesized to have reduced the abundance of young larvae in lower Shelikof Strait (Incze et al. 1989). In 1987, however, 66% of the drifters which left Shelikof Strait and headed down the sea valley towards offshore areas eventually returned to the continental shelf east of the Shumagin Islands. This suggests that some of the larvae which appeared to be headed for offshore areas may have returned to coastal waters.

Few walleye pollock were found offshore of the 200-m depth contour in the June–July 1987 survey. This indicates that either (1) fish carried out into these areas from Shelikof Strait do not survive, (2) they are carried back onto the shelf, or (3) they are entrained in the Alaskan Stream and are rapidly carried westward beyond the survey area at rates up to 100 cm/s.

The fairly uniform distribution pattern of late larval and early juvenile walleye pollock made the calculation of total abun-

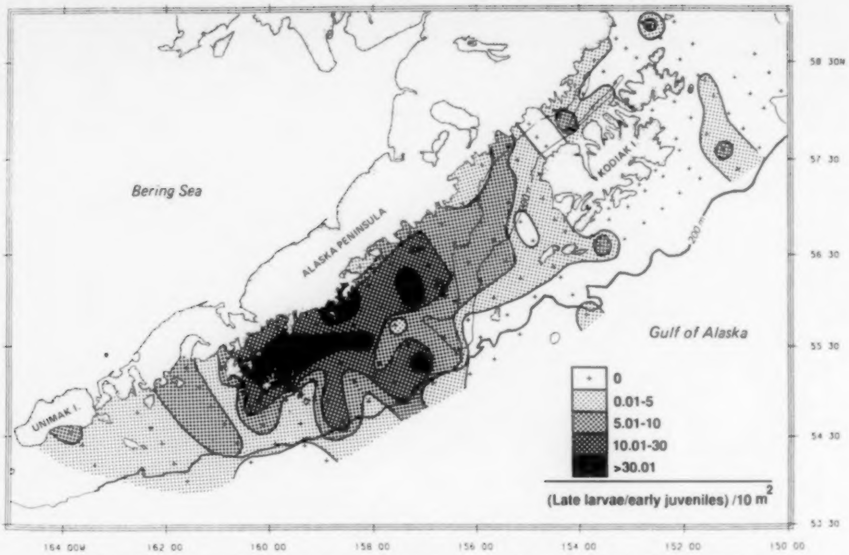
Bering Sea
ALASKA PENINSULA
KODIAK I.
UNIMAK I.
Gulf of Alaska
200 m
+ 0
0.01-5
5.01-10
10.01-30
>30.01
(Late larvae/early juveniles) /10 m²
58 30N
57 30
56 30
55 30
54 30
53 30
164 00W
162 00
160 00
158 00
156 00
154 00
152 00
150 00

FIG. 6. Distribution of late larval and early juvenile walleye pollock in June and July 1987.

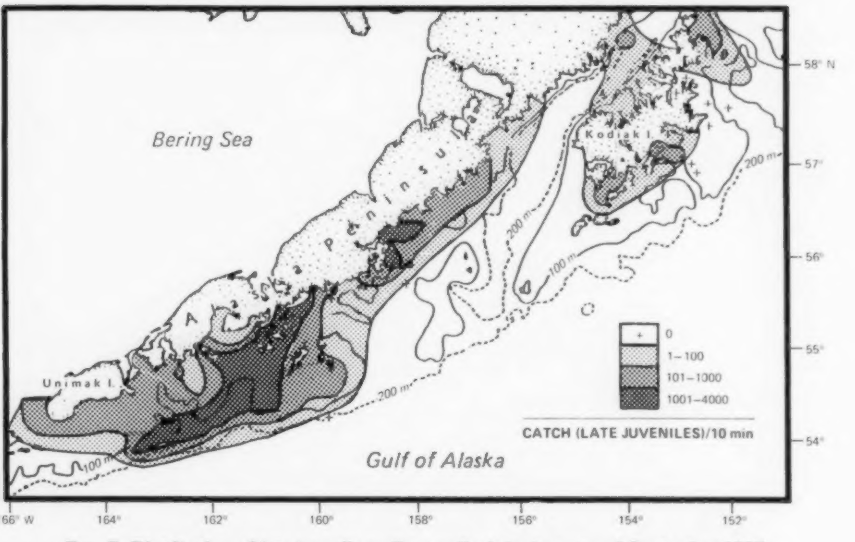
Bering Sea
Alaska Peninsula
Kodiak I.
Unimak I.
Gulf of Alaska
200 m
100 m
+ 0
1–100
101–1000
1001–4000
CATCH (LATE JUVENILES)/10 min
58° N
57°
56°
55°
54°
166° W
164°
162°
160°
158°
156°
154°
152°

FIG. 7. Distribution of late juvenile walleye pollock in August and September 1987.

dance relatively straightforward, as variances among stations within strata were relatively low. This is in contrast with the patchy distribution of older juveniles in August and September, by which time schooling has probably begun (B. Olla, Hatfield Mar. Sci. Center, Newport, OR 97365, pers. comm.) and behavior has become an important factor affecting distribution, making them more difficult to sample quantitatively.

Although we applied no correction factor for escapement of small larvae, or avoidance of the net by larger larvae, comparisons of the Methot trawl with a smaller-mesh trawl (S. Hinckley, unpubl. data) show that during this survey, escapement of smaller larvae was minimal. There remains the possibility of larger larvae avoiding the trawl, which would have caused an underestimate of total abundance, and which should be investigated. The results of this study indicate that the late larval and early juvenile stages may be appropriate for a prerecruit survey due to distributional features and ease of capture, if their abundance can be correlated with recruitment.

The results we have presented are important towards developing our understanding of walleye pollock recruitment in the western Gulf of Alaska. In 1987, about 90% of the late larvae and early juveniles in the western Gulf of Alaska were found downstream of Shelikof Strait, and our evidence indicates that they originated from the spawning there. Spawning in areas other than Shelikof Strait, for example, to the northeast of Kodiak Island, appeared to contribute very little to the total abundance of late larvae and early juveniles in the western Gulf of Alaska in 1987. This agrees with Kendall and Picquelle (1990) who found that, between 1981 and 1986, less than 11% of the total walleye pollock eggs found in the western Gulf were in areas other than Shelikof Strait. Unless 1987 was an anomalous year, this study supports the concept that recruitment in

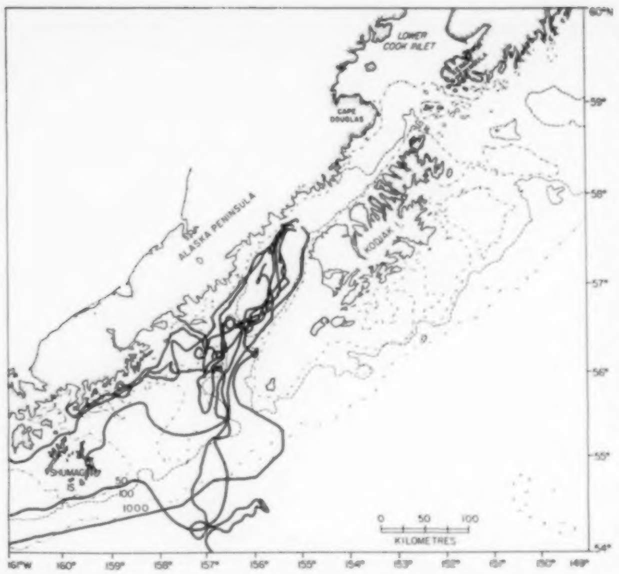

FIG. 8. Trajectories from seven satellite-tracked drifting buoys which show the generally southwestward flow. The buoys which grounded near the Shumagin Islands arrived at about the same time (June 1987) that high abundances of late larval walleye pollock were found. (Reprinted from Reed et al. 1988).

TABLE 1. Comparison of mean catch rates (number of larvae per 10 m^2) of Methot trawl for day stations and night stations for the nine strata (sample sizes in parentheses).

Strata	Day	Night
1	0.627 (3)	0.458 (4)
2	6.95 (11)	24.4 (4)
3	17.5 (10)	27.8 (7)
4	12.4 (12)	14.9 (7)
5	0.941 (13)	3.06 (8)
6	0.352 (13)	0.775 (6)
7	0.134 (21)	0.344 (8)
8	0.026 (8)	0.304 (5)
9	0.219 (7)	0.00 (2)
Mean	4.07 (97)	7.48 (51)

the western Gulf of Alaska depends largely on the spawning in Shelikof Strait.

We believe that the western Gulf of Alaska walleye pollock stocks are analogous in many ways to Arcto-Norwegian cod (*Gadus morhua*). The ACC and the Norwegian Coastal Current are similar in mean current speeds (10–60 cm/s) and estimated baroclinic transport (0.5–1.0 × 10^6 m/s, Reed et al. 1989; Schumacher et al. 1989; Saetre and Ljøen 1972). Flow conditions determined by the interactions between bathymetric features and currents dominate the local circulation in both systems (Schumacher 1987). Fish spawn in restricted areas in both systems (Kendall et al. 1987; Ellertsen et al. 1984). The peak of spawning in both areas is mid-March to mid-April and is similar in its duration and stability of timing (Kendall et al. 1987; Bergstad et al. 1987). The distribution of Arcto-Norwegian cod and of walleye pollock appears to be influenced at all stages by the local coastal current system (Schumacher 1987) with drift of eggs and planktonic larvae to a downstream juvenile nursery area. The rate of advection appears to depend on the strength and variability of the coastal current, as affected by eddies, bottom topography, and wind stress (Schumacher 1987; Ellertsen et al. 1984). Arcto-Norwegian cod and walleye pollock are also similar in that the late larval stages are pelagic and are not yet schooling (Sundby et al. 1989), making abundance estimation less complicated than at the later juvenile (0 group) stage.

Prerecruit surveys of postlarval Arcto-Norwegian cod have been shown to correlate well with the abundance of 3-yr-old cod and have been used as an early index of recruitment (Sundby et al. 1989). If this holds true for walleye pollock (and we cannot yet assess this with only 1 yr of data on this stage), then a prerecruit monitoring program of the late larval and early juvenile stages of walleye pollock could provide a timely index of year-class strength for fisheries managers. At present, managers get their first quantitative information on the strength of the incoming year class from the hydroacoustic estimate of 3-yr-old fish in March of the year they recruit into the fishery.

Transport of eggs and larvae to an appropriate juvenile nursery area has long been thought to affect year-class strength (Hjort 1914). Factors which result in disturbance of the optimal transport regime may affect recruitment of that year class. Many studies have proposed mechanisms by which transport affects survival (Nelson et al. 1977; Bailey 1981; Sinclair et al. 1985; see also review in Norcross and Shaw 1984); however, as mentioned by Wooster and Bailey (1989), it is difficult to demonstrate the negative effects of transport into unfavorable areas. Studies showing egg and larval transport by currents have been done (Kendall et al. 1987; Fortier and Leggett 1985), but few follow a cohort through the juvenile stage as does this study. Transport, however, is only one condition affecting recruitment, and it is probably not realistic to assume that it is a deterministic cause of year-class success or failure.

Acknowledgements

We thank A. Kendall, J. Traynor and the AFSC Hydroacoustics group, H. Shippen, and L. Incze for making preliminary data available and A. Brown and N. Merati for able technical assistance.

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A Small RNA Virus Isolated from Salmonid Fishes in California, USA

R. P. Hedrick[1] and S. Yun

Department of Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

and W. H. Wingfield

California Department of Fish and Game, Fish Disease Laboratory, Rancho Cordova, CA 95670, USA

Hedrick, R. P., S. Yun, and W. H. Wingfield. 1991. A small RNA virus isolated from salmonid fishes in California, USA. Can. J. Fish. Aquat. Sci. 48: 99–104.

Beginning in the summer of 1988, examinations of numerous salmonid broodstocks in northern California, USA, revealed widespread infections with a previously undescribed virus. The virus was isolated from ovarian fluids of adult rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*O. clarki*), brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis*) and from kidney and spleen samples of juvenile brown and brook trout. The virus was not associated with above-normal losses in adult or juvenile fish. Virions purified from infected CHSE-214 cells were hexagonal with a mean diameter of 37.5 nm (SD = 0.41) and did not possess an envelope. The virus induced a diffuse degenerative cytopathic effect in CHSE-214 cells 14–28 d after inoculation with ovarian fluids or tissue homogenates. The virus replicated in CHSE-214 cells at temperatures from 10 to 20°C with an optimum at 15°C. Replication of the virus was not inhibited by addition of 5-bromo-2-deoxyuridine (BUDR) to the growth medium. There was no virus-induced mortality but virus was recovered for periods of up to 3–5 wk following waterborne exposures of rainbow and brown trout and kokanee salmon (*O. nerka*) but not from chinook (*O. tshawytscha*) or coho salmon (*O. kisutch*).

Depuis l'été 1988, on a observé que de nombreux stocks de reproducteurs de salmonidés du nord de la Californie, É.-U., étaient largement infectés par un virus jusqu'alors inconnu. Ce virus a été extrait des fluides ovariens de truites arc-en-ciel (*Oncorhynchus mykiss*), de truites fardées (*O. clarki*), de truites brunes (*Salmo trutta*) et d'ombles de fontaine (*Salvelinus fontinalis*) adultes de même que d'échantillons regroupant les reins et la rate prélevés sur des truites brunes et des ombles de fontaines juvéniles. La présence de ce virus n'augmente pas la mortalité chez les adultes et les juvéniles. Les virions purifiés tirés de cellules CHSE-214 infectées étaient hexagonaux, leur diamètre moyen était de 37,5 nm (écart-type = 0,41) et étaient dépourvus d'enveloppe. Le virus induisait un effet cytopathologique diffus dans des cellules CHSE-214 14 à 28 jours après inoculation de fluides ovariens ou d'homogénats de tissus. Le virus pouvait se répliquer dans ces cellules à des températures allant de 10 à 20°C, la température optimale étant de 15°C. L'addition de 5-bromo-2-désoxyuridine (BUDR) au milieu de culture n'inhibait pas la réplication du virus. L'exposition au virus dans l'eau n'a pas entraîné de mortalité; toutefois, le virus a été détecté en 3 à 5 sem chez les truite arc-en-ciel et brune de même que chez le saumon kokanee (*O. nerka*), mais pas chez les saumons quinnat (*O. tshawytscha*) et coho (*O. kisutch*).

Received February 19, 1990
Accepted July 23, 1990
(JA479)

Reçu le 19 février 1990
Accepté le 23 juillet 1990

Although most families of animal viruses are represented in fish (Wolf 1988), there are only four reports of small (<40 nm in diameter) RNA viruses having been isolated from fish. These have often been referred to as "picorna-like" viruses because of their small size, apparent RNA genome, and lack of an external envelope, features consistent with the family picornaviridae (Appleton 1987; Oliver and Phillips 1988).

Two of the four small RNA fish viruses were isolated from smelt. Moore et al. (1988) isolated an agent they described as picorna-like from rainbow smelt (*Osmerus mordax*) in New Brunswick, Canada. The smelt virus (SV) was 20–30 nm in diameter and hexagonal in shape. A virus with identical properties to SV was isolated by Ahne et al. (1990) from European smelt (*O. eperlanus*) in the Elbe River estuary, Federal Republic of Germany. Although the two smelt viruses induced different cytopathic effects (CPE) in salmonid cell lines, they shared a similar size, morphology, lack of an envelope, and resistance to DNA inhibitors (Moore et al. 1988; Ahne et al. 1990). The SV replicated in the tissues of juvenile brook trout (*Salvelinus fontinalis*), in the absence of disease, that received intraperitoneal injections with the virus (Moore et al. 1988).

A third small RNA virus (ASV) was recovered from Atlantic salmon (*Salmo salar*) in Washington State, USA (Hedrick et al. 1990). This virus shares similar physical properties to the smelt viruses and induces syncytia in CHSE-214 cells as reported for SV.

The fourth small RNA virus reported from fish has been placed into the family Caliciviridae and is designated San Miguel sea lion virus (SMSV). The virus was isolated from the marine fish opaleye (*Girella nigricans*) and replicates in VERO cells from the African green monkey (*Cercopithecus aethiops*) incubated at 37°C (Smith et al. 1980). The virus was found to induce a vesicular exanthema in experimentally infected pinnipeds (Smith et al. 1980). Several serotypes of SMSV have been isolated from California sea lions (*Zalophus californianus*), northern fur seals (*Callorhinus ursinus*), and northern elephant seals (*Mirounga agustirostris*) (Smith et al. 1980). Caliciviruses can be distinguished from other small RNA viruses by their larger size (35–39 nm) and presence of "cupped shaped" depressions on the capsid surface (Cubitt et al. 1987). No such features were reported for the smelt viruses (Moore et al. 1988; Ahne et al. 1990) or ASV (Hedrick et al. 1990). The lack of this characteristic morphology and a failure to replicate in mammalian cell lines suggest that these picorna-like agents are not typical caliciviruses but instead novel viruses from fish.

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[1] Author to whom correspondence should be directed.

In this report we describe a fifth small RNA virus isolated from several species of salmonids reared in northern California, USA. Although the virus did not cause mortality, it was found to replicate in experimentally exposed salmonid fishes. This property and the occurrence of the virus in numerous broodstocks caused us to examine in more detail the characteristics of this new virus.

Materials and Methods

Virus Isolation

Beginning in the summer of 1988 and continuing to the present, numerous salmonid broodstocks in northern California were examined for the presence of virus. Ovarian fluids were collected from female rainbow (*Oncorhynchus mykiss*), cutthroat (*O. clarki*), brown (*Salmo trutta*), and brook trout at the time of spawning and inoculated onto CHSE-214 and EPC cells using standard methods (Amos 1985). The CHSE-214 and EPC lines were propagated using standard methods in minimal essential medium (MEM) supplemented with 50 units penicillin/mL, 50 μg streptomycin/mL and 7.5% fetal bovine serum at 20°C (Amos 1985).

Cells inoculated with ovarian fluids were incubated at 15°C and observed for CPE over a 28-d period. In later examinations, juvenile brook and brown trout were examined for virus by inoculation of tissue homogenates made from portions of the kidney and spleen from five fish pools as described by Amos (1985). When CPE was observed, two successive subcultures (1:50 dilutions) were inoculated onto CHSE-214 cells. Virus harvested from the cell culture fluid of these cells was then divided into aliquots and stored at −70°C. The virus isolated from ovarian fluids of adult cutthroat trout from Heenan Lake (CTV) was used for all subsequent studies.

Electron Microscopy

Virus was grown in sixteen 150-cm^2 flasks containing CHSE-214 cells at 15°C until CPE were well advanced. Virus was isolated from the supernatant and cell debris by the method described by Hedrick et al. (1983). Partially purified virus was resuspended in 1% phosphotungstic acid, observed, and photographed with a Philips EM 400 electron microscope.

Growth Temperature Study

CHSE-214 cells in 25-cm^2 flasks containing 5 mL of MEM supplemented with 2% fetal bovine serum (MEM-2) were inoculated with $10^{3.5}$ 50% tissue culture infectious dose ($TCID_{50}$) of the virus. Two flasks were then incubated at 4, 10, 15, 20, and 25°C. At 3, 5, 9, 13, 19, and 29 d after inoculation, 0.1 mL of medium was removed from each flask. The concentrations of virus present were determined by $TCID_{50}$ analyses on CHSE-214 cells incubated at 15°C for 21 d and expressed as the average $TCID_{50}$ per millilitre of growth medium. Growth medium from uninoculated CHSE-214 cells was sampled at 29 d as a cell control. The ability of the virus to induce CPE in a mammalian cell line was also tested at 37°C by inoculation of a monolayer of VERO cells in a 25-cm^2 flask with $10^{3.9}$ $TCID_{50}$ of the salmonid virus.

Nucleic Acid Inhibitor Study

To determine the nucleic acid type of the new virus, the sensitivity of virus replication was examined in the presence of the DNA inhibitor 5-bromo-2-deoxyuridine (BUDR) with and without thymidine. Duplicate 25-cm^2 flasks of CHSE-214 cells contained either MEM-2 only, MEM-2 plus 50 μg BUDR/mL, or 50 μg BUDR/mL plus 50 μg thymidine/mL. The flasks were inoculated with $10^{3.0}$$TCID_{50}$ of virus and incubated at 15°C, and the concentration of virus was determined by $TCID_{50}$ analyses on CHSE-214 cells at 15°C after 21 d. *Oncorhynchus masou* virus (OMV) (Kimura et al. 1981) and infectious pancreatic necrosis virus (IPNV), strain VR-299 (Wolf et al. 1960), were used as controls for DNA and RNA viruses, respectively.

Stability of the Virus

The stability of CTV was examined at 15, 37, 45, 50, 55, and 60°C by incubation of 2.0 mL of culture medium from infected CHSE-214 cells at each temperature. The average concentration of virus remaining after 15 min of incubation in replicate tubes at each temperature was determined by $TCID_{50}$ analysis on CHSE-214 cells incubated at 15°C for 21 d.

Chloroform Sensitivity

Two-millilitre stock suspensions of virus were treated with 10% chloroform overnight at 4°C. The aqueous portion was harvested after centrifugation at 1000 × *g* for 10 min. The concentrations of virus were then determined for both treated and untreated (no chloroform but an equal volume of MEM-2) by $TCID_{50}$ analyses on CHSE-214 cells incubated at 15°C after 21 d. Two control viruses, IPNV and infectious hematopoietic necrosis virus (IHNV) (Amend and Chambers 1970), were tested under the same conditions, and concentrations of virus were determined by $TCID_{50}$ analyses on CHSE-214 cells incubated at 15°C for 21 d.

Neutralization Comparisons

The potential relationship of the new virus to IPNV, IHNV, and *Herpesvirus salmonis* virus (HPV) (Wolf et al. 1978) was examined with hyperimmune rabbit serums produced against the three viruses. Neutralization indices (the difference in titer between virus without and with antisera) were calculated with each antiserum against the homologous virus and CTV (Rovozzo and Burke 1973).

Fish Exposures

A trial was conducted to determine the effects of the new virus on several species (mean weight) of salmonids including rainbow (1.2 g) and brown trout (0.7 g) and kokanee (0.26 g), coho (*O. kisutch*) (0.51 g), and chinook salmon (*O. tshawytscha*) (0.45 g). Groups of 100 fish were exposed to $10^{5.6}$ $TCID_{50}$ virus/mL in a 20-L aquarium. After 1 h, the flow of 15°C well water was resumed to each aquarium. An equal size control group of each species was exposed to MEM-2 only. At either 1, 3, 4, or 6 wk following exposure, the kidney and

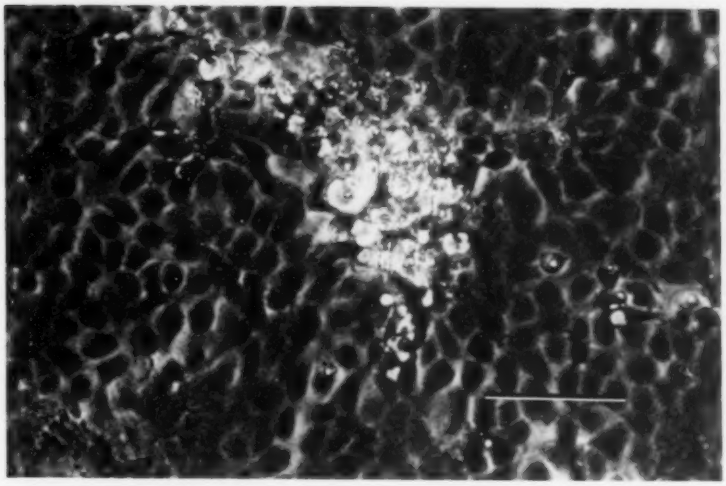

FIG. 1. CPE induced in CHSE-214 cells 7 d following inoculation with CTV. Bar = 30 μm.

spleen from individual fish were examined for the presence of virus by standard procedures on CHSE-214 cells at 15°C (Amos 1985).

In a second trial, 100 rainbow trout (mean weight 0.6 g) were exposed to $10^{7.4}$ $TCID_{50}$ virus/mL for 1 h. A parallel control group was exposed under the same conditions but only to cell culture fluid with no virus. The water temperature was 15°C. Ten fish were removed from the experimental and control groups at 3 d and 1, 2, 3, and 4 wk following exposure and examined for concentrations of the virus in a tissue sample containing both kidney and spleen. Virus concentrations were determined by $TCID_{50}$ analysis on CHSE-214 cells incubated at 15°C for 4 wk. At each sampling time, five fish were fixed in Davidson's solution (Humason 1979), embedded in paraffin, sectioned to 5 μm, and stained with hematoxylin and eosin.

Results

Isolation of the Virus

CPE were observed at 28 d after infection of CHSE-214 cells at 15°C with ovarian fluids from several salmonid broodstocks from northern California. The cytopathology was characterized by initial focal areas of cell rounding and necrosis that later spread to involve but not completely destroy the surrounding monolayers (Fig. 1). Virus isolates were obtained from every major rainbow broodstock examined in northern California and also two brown trout, one brook trout, and one cutthroat trout broodstock. Juveniles reared at two locations with virus-positive brown trout and the brook trout broodstocks were found to harbor the virus when examined 1 mo after hatching.

Electron Microscopy

Virions could be partially purified from infected cells using extraction procedures described for birnaviruses combined with CsCl gradient centrifugation (Hedrick et al. 1983). The virus formed a band at a density of approximately 1.34 g/mL in CsCl gradients. Electron microscopy showed that the band contained numerous round to icosahedral shaped virions with a mean diameter of 37.5 nm (x = 20, SD = 0.41). Many particles appeared to be empty shells (Fig. 2).

Growth Temperature

Virus was detected in the supernatant of CHSE-214 cultures at 10, 15, and 20°C at 3 d postexposure (Table 1). At 13 d, virus was detected at 4°C but was never detected at 25°C. The virus grew well at 10, 15, and 20°C, the greatest virus concentration being obtained at 19 d at 15°C. Replication at 4°C was slow but at 29 d, virus concentrations were still increasing. No evidence of virus replication was detected at 37°C in VERO cells inoculated with the virus after 7 d of observation.

Nucleic Acid Type

There was only a slight reduction (0.7 $TCID_{50}$/mL) in the concentration of CTV following treatment of CHSE-214 cells with BUDR (Table 2). The replication of the control RNA virus IHNV was also not affected by the presence of BUDR in the medium (Table 2). In contrast, the DNA virus HPV was completely inhibited by the presence of BUDR.

Stability of the Virus

The concentration of the virus was not affected by incubation at 37°C compared with 15°C for 15 min (Table 3). The virus was progressively less stable as temperatures increased but 1000 $TCID_{50}$ virus/mL remained after 15 min at 60°C.

Chloroform Sensitivity

The infectivity of CTV and IPNV were not substantially reduced following treatments with chloroform (Table 4). In contrast, IHNV infectivity was completely lost when so treated.

Neutralization Studies

None of the hyperimmune rabbit sera produced against reference viruses from salmonid fishes substantially neutralized the CTV (Table 5). The greatest reduction in CTV titer was observed with IHNV antisera ($10^{1.2}$ $TCID_{50}$). This level, however, was well below the reduction observed with the same antisera against IHNV ($10^{4.0}$ $TCID_{50}$).

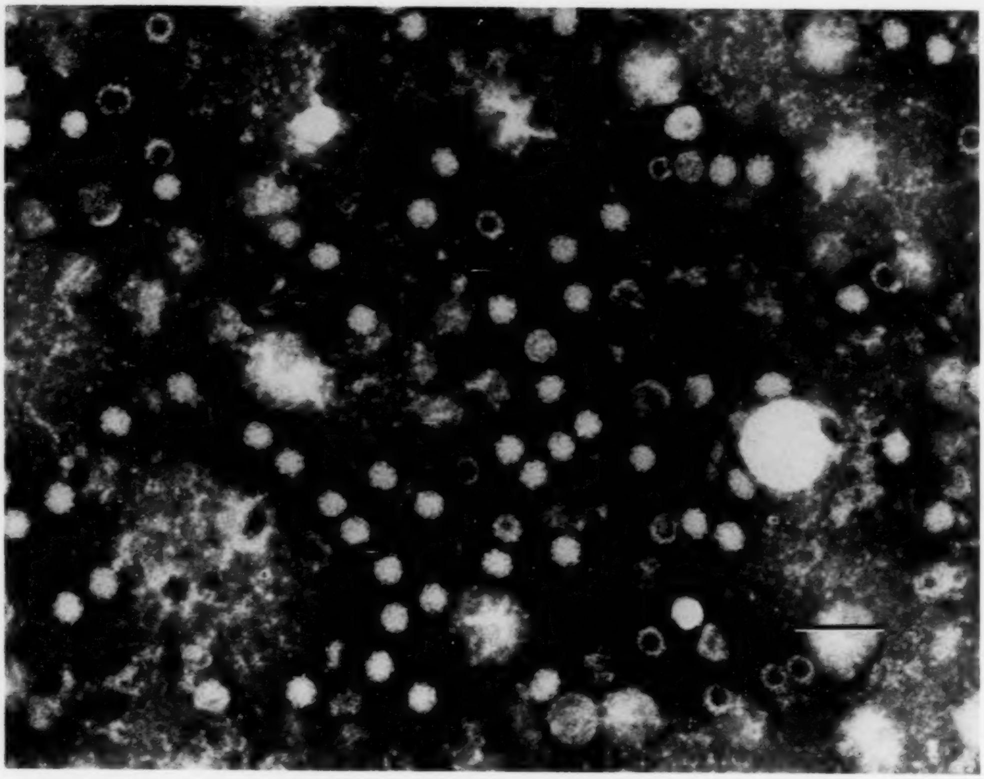

FIG. 2. Electron micrograph of CTV as partially purified from infected CHSE-214 cells. Bar = 100 nm.

TABLE 1. Concentrations ($\log_{10}$ TCID$_{50}$) of CTV in the CHSE-214 cell line at selected temperatures. Replicate flasks of CHSE-214 cells were inoculated with $10^{2.9}$ TCID$_{50}$ of virus. Two flasks were then placed at each temperature for incubation. At selected times, 0.1 mL of culture medium was removed and the average concentrations of virus determined by TCID$_{50}$ analyses on CHSE-214 cells at 15°C for 21 d.

	Temperature (°C)				
Days	4	10	15	20	25
3	—[a]	1.9	2.8	3.7	—
5	—	3.1	4.6	5.1	—
9	—	5.3	7.0	7.3	—
13	2.8	7.5	7.8	7.5	—
19	4.9	8.1	8.9	7.6	—
29	6.8	8.9	8.5	5.9	—

[a] No virus detected at lowest dilution examined (10^{-1}).

TABLE 3. Stability of CTV following incubation at selected temperatures. Two tubes containing 2.0 mL of culture fluid from infected CHSE-214 cells were incubated at each temperature. After 15 min, the average concentration of virus was determined by TCID$_{50}$ analyses on CHSE-214 cells incubated at 15°C for 21 d.

Temperature (°C)	$\log_{10}$ TCID$_{50}$/mL
15	7.9
37	7.8
45	6.2
50	4.1
55	3.3
60	3.0

TABLE 2. Sensitivity of CTV replication in the presence of BUDR. The sensitivity of three viruses, CTV, IPNV, and OMV were examined without inhibitor, with 50 μg BUDR/mL, and with BUDR plus 50 μg thymidine/mL.

	Virus concentration ($\log_{10}$ TCID$_{50}$/mL)		
Virus	MEM-2	MEM + BUDR	MEM + BUDR + thymidine
CTV	8.2	7.3	7.2
IPNV	10.0	9.5	9.7
OMV	5.8	—[a]	2.9

[a] No virus detected at lowest dilution examined (10^{-1}).

TABLE 4. Sensitivity of CTV to treatments with chloroform. Concentrations of CTV, IPNV, and IHNV detected in virus suspensions either untreated or treated with 10% chloroform overnight at 4°C.

	$\log_{10}$ TCID$_{50}$/mL		
Virus	Control	Treated	Difference
CTV	8.1	8.0	0.1
IPNV	8.8	8.8	0.0
IHNV	8.1	—[a]	8.1

[a] No virus detected at lowest dilution examined (10^{-1}).

TABLE 5. Effect of antisera against IPNV, IHNV, and HPV on CTV, on cutthroat trout virus (CTV). The concentration of each control virus was determined in the presence and absence of homologous antiserum. In parallel tests, the effect of each antiserum on CTV was examined. Neutralization indices are expressed as the $\log_{10}$ $TCID_{50}$ difference between the concentrations of virus with and without antiserum added (Rovozzo and Burke 1973).

	Log_{10} neutralization indices ($TCID_{50}$/mL)	
Virus	Control virus	CTV
IPNV	6.5	0
IHNV	4.0	1.2
HPV	2.3	0.5

TABLE 6. Susceptibility of selected salmonids to waterborne exposure to CTV. Groups of 100 fish were exposed to $10^{5.6}$ $TCID_{50}$ virus/mL for 1 h and then held in 15°C well water in 20-L aquaria.

		Weeks postexposure (no. virus positive/no. tested)			
Species	Mean fish weight (g)	1	3	4	6
Rainbow trout	1.20	5/5[a]	—	0/5	—
Chinook salmon	0.45	0/5	—	0/5	—
Coho salmon	0.57	0/5	—	—	—
Kokanee salmon	0.26	2/2	—	5/5	—
Brown trout	0.70	—	5/5	—	5/5

[a] No mortalities occurred during the study but on sampling dates, five fish were removed and the kidney and spleen of individual fish were combined and the concentration of virus determined by $TCID_{50}$ analyses on CHSE-214 cells incubated at 15°C for 21 d.

TABLE 7. Concentrations ($\log_{10}$ $TCID_{50}$/g) of virus found in the kidney and spleen of juvenile rainbow trout following waterborne exposure to CTV. Ten fish (mean weight 5.6 g) were examined at each sampling. The spleen and kidney from individual fish were combined and then the concentration of virus determined by $TCID_{50}$ analyses on CHSE-214 cells incubated at 15°C for 21 d after inoculation with suspensions from virus-exposed fish.

	Days				
Sample No.	3	7	14	21	28
1	6.0	4.0	2.9	3.6	3.8
2	5.4	3.9	2.6	0	3.8
3	4.8	3.7	—[a]	0	3.8
4	4.7	3.6	0	0	3.6
5	4.7	0	0	0	0
6	4.5	0	0	0	0
7	4.2	0	0	0	0
8	3.7	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0

[a] Sample was positive, but titer could not be determined.

Fish Exposures

In the first trial, the virus was recovered from all rainbow trout and kokanee salmon examined 1 wk after exposure to the virus (Table 6). By the second week, virus was found in brown and brook trout (not examined at week 1). Virus was also detected in all kokanee salmon at 4 wk and all brown trout at 6 wk (Table 6). Concentrations of the virus were not determined but based on the dilution of tissue homogenates (1:100 w/v), a minimum of 100 $TCID_{50}$ virus/g was present in all samples where virus was detected. Examinations of tissue sections from fish at each sampling time failed to show any microscopic pathology that differed from that observed in control fish. No virus was detected in 10 control fish of each species examined at 1, 3, and 6 wk.

In the second trial with rainbow trout, the concentrations of virus found in a pooled sample of kidney and spleen tissue from individual fish were determined (Table 7). Concentrations of virus of $10^{3.7-6.0}$ $TCID_{50}$/g were detected in 8 of 10 trout examined 3 d after exposure to the virus. Virus was detected in fewer fish and at lower concentrations in specimens examined at 1, 2, 3, and 4 wk postexposure. At 4 wk, however, 4 of 10 fish still harbored virus (Table 7). No virus was detected in 5 controls examined at each sampling time.

Discussion

Small RNA viruses were detected in 10 major rainbow trout, two brown trout, one cutthroat trout, and one brook trout broodstock in northern California. Because the virus was first detected in adult cutthroat trout, it has been designated cutthroat trout virus or CTV.

This newly recognized virus shares certain characteristics with four previously reported agents recovered from fish. The size and shape of CTV most closely resembles SMSV as described by Smith et al. (1980). The inability to replicate at temperatures above 20°C and in VERO cells at 37°C, however, clearly separates CTV from SMSV.

The three other small RNA viruses described from fish share the common features of replication in a salmonid cell line (CHSE-214) at low temperatures but not in mammalian lines at 37°C (Moore et al. 1988; Ahne et al. 1990; Hedrick et al. 1990). These viruses may be distinguished, however, by their size and the CPE induced in CHSE-214 cells. Both Moore et al. (1988) and Ahne et al. (1990) reported diameters between 20 and 30 nm for the smelt viruses. This contrasts with 39.5 and 37.5 nm for ASV (Hedrick et al. 1990) and CTV, respectively. Although size would tend to suggest a relationship between CTV and ASV, the latter induces syncytia in CHSE-214 cells, a property also shared by SV (Moore et al. 1988). Descriptions of the CPE for the second smelt virus by Ahne et al. (1990) include a slowly developing diffuse necrosis identical to that observed with CTV (Fig. 1).

The four small RNA viruses from smelt and salmonids appear to be somewhat related and may represent a previously unrecognized group of viruses from fish for which no suitable taxonomic characteristics apply. Replication of the viruses in a salmonid cell line with optimal temperatures near 15°C suggests that all four viruses are indeed from poikilothermic species.

The most comprehensive examinations of the potential effects of the viruses on fish have been conducted with ASV (Hedrick et al. 1990) and CTV as presented in this study. No evidence for virus replication was demonstrated in Atlantic salmon or rainbow trout exposed to ASV (Hedrick et al. 1990), and attempts to reisolate the virus from the original population of fish were unsuccessful. In contrast, CTV reached concentrations up to 10^6 $TCID_{50}$/g of tissues of rainbow trout exposed to the virus in the water (Table 7). In addition, CTV has been found in several salmonid broodstocks (including recent isolations from rainbow trout in Oregon and Utah). However, in neither naturally occurring or experimental infections with CTV were any adverse effects observed in the fish.

The greatest impact of the virus has been on movements of fish and fish eggs between states and countries. Although it is impractical to investigate the effects of CTV on all species of fish, further studies on the potential host range and transmission with ova from infected adults are needed and are under investigation in our laboratory. Similar host range studies with the smelt viruses combined with biochemical and serological comparisons with all of the small RNA viruses of fish should provide further insights into this newly recognized group of fish viruses.

Acknowledgements

The authors wish to thank Dr. J. M. Groff, Mr. R. Munn, and the histotechnicians at the Veterinary Medicine Teaching Hospital for their technical assistance.

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Biomass Size Spectrum of the Lake Michigan Pelagic Food Web[1]

W. Gary Sprules
Department of Zoology, Erindale College, University of Toronto, Mississauga, Ont. L5L 1C6, Canada
S. B. Brandt
University of Maryland, Chesapeake Biological Laboratory, Solomons, MD 20688, USA
D. J. Stewart
State University of New York College of Environmental Science and Forestry, 256 Illick Hall, Syracuse, NY 13210, USA
M. Munawar
Department of Fisheries and Oceans, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6, Canada
E. H. Jin
Department of Zoology, Erindale College, University of Toronto, Mississauga, Ont. L5L 1C6, Canada
and J. Love
University of Maryland, Chesapeake Biological Laboratory, Solomons, MD 20688, USA

Sprules, W. G., S. B. Brandt, D. J. Stewart, M. Munawar, E. H. Jin, and J. Love. 1991. Biomass size spectrum of the Lake Michigan pelagic food web. Can. J. Fish. Aquat. Sci. 48: 105–115.

Biomass size spectra for the complete Lake Michigan pelagic food web from picoplankton to salmonids were constructed for nine sampling transects around the lake in May and in September 1987. Size spectra were typical for freshwaters, having distinct peaks corresponding to major size groups. Biomass concentration of algae, zooplankton, and planktivores conformed to particle-size model predictions, but piscivore biomass was lower than predicted because these species are stocked. Mean annual total pelagic biomass was 72.3 $\mathrm{g \cdot m^{-2}}$ compared with a predicted range of 78.8–85.3 $\mathrm{g \cdot m^{-2}}$. Potential production of piscivores, *Mysis*, and *Pontoporeia* was in agreement with model predictions. No estimates of zooplankton or planktivore production were available, but we calculated that these could be 72.1–91.6 and 2.5–4.1 $\mathrm{g \cdot m^{-2} \cdot yr^{-1}}$, respectively. Our analyses suggest that piscivore production is constrained by food web structure. Bloater, which comprise 72% of planktivore biomass, make up less than 20% of salmon diets. We estimate that piscivore production could be double the current value of 0.27 $\mathrm{g \cdot m^{-2} \cdot yr^{-1}}$ if the forage fish community changed to include species more available to salmon.

La gamme des dimensions biomassiques de tout le réseau trophique pélagique du lac Michigan, du picoplancton jusqu'aux salmonidés, a été établie pour neuf transects d'échantillonnage dans la région du lac, en mai et septembre 1987. La gamme des dimensions était caractéristique de celle des eaux douces et montrait des sommets nets correspondant aux principaux groupes de dimensions. La concentration biomassique des algues, du zooplancton et des planctonivores a suivi les prévisions du modèle granulométrique, mais la biomasse piscivore était plus faible que prévu, car ces espèces sont ensemencées. La moyenne annuelle totale de la biomasse pélagique était de 72,3 $\mathrm{g \cdot m^{-2}}$, comparativement à l'ordre de grandeur prévu de 78,8 à 85,3 $\mathrm{g \cdot m^{-2}}$. La production possible des piscivores, *Mysis* et *Pontoporeia* concordait avec les prévisions du modèle. On ne disposait d'aucune donnée estimative sur la production de zooplancton ou de planctonivores, mais nous avons situé cette production entre 72,1 et 91,6 $\mathrm{g \cdot m^{-2} \cdot année^{-1}}$ approximativement pour le zooplancton, et entre 2,5 et 4,1 $\mathrm{g \cdot m^{-2} \cdot année^{-1}}$ environ pour les planctonivores. Les résultats de nos analyses portent à croire que la production piscivore est limitée par la structure du réseau trophique. Le cisco de fumage, qui représente 72 % de la biomasse planctonivore, constitue moins de 20 % de l'alimentation du saumon. Nous croyons que la production piscivore pourrait être deux fois plus importante que celle qui est actuellement évaluée à 0,27 $\mathrm{g \cdot m^{-2} \cdot année^{-1}}$ si la communauté de poissons fourrage se modifiait de façon à inclure des espèces plus accessibles au saumon.

Received May 3, 1990
Accepted August 30, 1990
(JA564)

Reçu le 3 mai 1990
Accepté le 30 août 1990

The biomass size spectrum of an aquatic ecosystem is the distribution of living biomass across the range of organism size. Such spectra are tractable summaries of complex communities comprising hundreds of species representing many trophic groups. They have provided insight into various ecological processes in marine and freshwater ecosystems, including structure (Sheldon et al. 1972; Schwinghamer 1981; Peters 1983; Rodriguez and Mullin 1986; Sprules and Munawar 1986; Proft and Ronnenberger 1987; Hanson et al. 1989), potential fish production (Sheldon et al. 1977; Borgmann et al. 1984;

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[1] This paper is one of a series originating from a special session of the 32nd Conference on Great Lakes Research (Madison, WI, May 30 to June 2, 1989) entitled "Population Dynamics of Large Lake Ecosystems: Patterns, Processes, and Interlake Comparisons."

Leach et al. 1987; Minns et al. 1987), fisheries management (Pope et al. 1988), and size scaling of trophic interactions (Dickie et al. 1987).

An extensive theory of particle-size distributions based on size-dependent ecological and physiological processes has developed (Borgmann 1987). Its use at the scale of whole ecosystems is based on the premise that simple patterns observed for subsets of organisms (e.g. plankton) can be extrapolated to whole food webs (Sheldon et al. 1972, 1977). This can be confirmed only by obtaining temporally and spatially extensive biomass size data for all trophic groups in a community. No such data have yet been reported, although Hargrave et al. (1985) presented most of a pelagic marine spectrum averaged over time at a single station in St. Georges Bay, Gulf of St. Lawrence. In this paper we describe the complete Lake Michigan pelagic biomass size spectrum — including phytoplankton, zooplankton, planktivorous fish, and piscivorous fish — from nine major regions of the lake in both spring and summer 1987, compare these data with predictions of particle-size models, and estimate potential annual production of various trophic groups.

Materials and Methods

Data for plankton and planktivorous fish were collected during two research cruises on Lake Michigan exclusive of Green Bay, one in the spring from May 21 to 30 and one in the summer from August 27 to September 9, 1987. Nine onshore–offshore transects beginning at the 0- to 9-m depth contour and ending at the 90- to 110-m contour were sampled on each cruise (Fig. 1). Inshore plankton samples were collected (except as noted in Fig. 1) near the beginning of each transect at depths ranging from 9 to 35 m, while offshore plankton samples (except as noted in Fig. 1) were collected near the end of each transect at depths ranging from 91 to 175 m. Phytoplankton samples were collected with an integrating sampler (Schroeder 1969) hauled from depth to the surface. During the spring cruise these vertical hauls were taken from 20 m to the surface except at the shallow inshore sites at Manistique and Sturgeon Bay where they were taken from 7 m to the surface. During the summer cruise, all offshore phytoplankton hauls were taken from 36.5 m to the surface, and inshore ones were taken from 16.8 m to the surface. Phytoplankton samples were preserved in Lugol's solution and then settled and examined under an inverted microscope according to the Utermöhl technique (Munawar et al. 1974). All phytoplankton, including picoplankton, nanoplankton, and net plankton up to large cells and colonies, were included in the analyses. Organisms were identified to species and total biomass computed by using geometrical models of cell shape. Resultant biomass estimates were expressed in units of fresh mass per unit surface area (grams per square metre) assuming a specific gravity of 1 (Munawar et al. 1974). Comparison of biomasses measured in areal units requires that sampled water columns are of approximately uniform length. We thus extrapolated spring offshore 20-m phytoplankton samples up to the 36.6 m of summer offshore samples by assuming algal biomass concentration to be uniform with depth (Fahnenstiel and Scavia 1987a).

Zooplankton samples were collected by hauling a conical townet of 50 cm mouth diameter and 110-μm nylon mesh from depth to the surface. Filtering efficiency of 70% was assumed, a value known to be realistic on the basis of flowmeter measurements we have independently made using this net in Lake Michigan. Spring samples were collected during the day (except offshore Manistique which was sampled at night) with inshore hauls initiated near bottom at depths ranging from 6 to 25 m and offshore hauls initiated at midwater depths ranging from 50 to 100 m. Summer samples were collected exclusively at night with inshore hauls initiated near bottom at a depth of 16.7 m (except inshore Ludington which was 13.7 m) and offshore hauls initiated at midwater depths ranging from 88.4 to 91.4 m. Samples were preserved in 4% sugared Formalin (Haney and Hall 1973) and examined under a dissecting microscope interfaced with an electronic measuring device (Sprules et al. 1981). Organisms were identified to functional group (Sprules and Jin 1990), enumerated, and their maximum linear size recorded. Individual organism mass was computed from standardized length – fresh weight regressions (Sprules 1984). All rotifers and crustaceans, including the large predators *Mysis relicta*, *Leptodora kindtii*, and *Bythotrephes cederstroemii*, were enumerated. Protozoa were not sampled and some of the smaller rotifer species were not captured by the 110-μm plankton net. Resultant biomass estimates were expressed as fresh mass per unit surface area (grams per square metre). Although some spring offshore zooplankton tows were 50 m whereas corresponding summer ones were 91.4 m, areal biomass concentrations are nevertheless comparable because our data indicate that almost all of the biomass occurs in the upper 50 m.

We did not sample the benthic amphipod *Pontoporeia hoyi* but estimated its biomass as follows. Nalepa (1989) summarized estimates of *P. hoyi* biomass (grams dry weight per square metre) from 40 sampling stations in the southern third of Lake Michigan for 1981 and 1982 combined. We averaged his values from four major depth intervals (16 to >90 m) to derive an estimate of $2.8\ \mathrm{g\ dry{\cdot}m^{-2}}$ and converted this to $18.6\ \mathrm{g\ wet{\cdot}m^{-2}}$ by assuming that dry weight is 15% of fresh weight. *Pontoporeia hoyi* densities in Lake Michigan decreased by about 50% from 1980 to 1985 (MacDonald et al. 1990), so we halved our estimate to produce a final value of $9.3\ \mathrm{g\ wet{\cdot}m^{-2}}$. On the basis of typical size distributions of *P. hoyi* (MacDonald et al. 1990), we determined that this species would occupy five logarithmic mass intervals, each of which would contain $9.3/5 = 1.9\ \mathrm{g{\cdot}m^{-2}}$ (see Fig. 7).

Planktivorous fish data were collected along each transect using two techniques. The first employed a 120-kHz dual-beam echosounder (Biosonics® model 102) that was operated continuously along the length of each sampling transect at night (Brandt et al. 1991). This generated whole water column estimates of the density of fish ranging in size from approximately 12 to over 300 mm. The second technique involved bottom trawls that were deployed during daytime to capture fish at selected depth contours along each onshore–offshore transect. The bottom trawl had a 7.6-m headrope, 2-cm-bar mesh and a 6-mm-mesh liner in spring and a 11.9-m headrope, 15.5-m footrope, and 13-mm-mesh liner in summer (Hatch et al. 1981). These data were used to determine the proportionate species and size composition of the fish assemblage. During the summer cruise, acoustic estimates of fish densities were not made at the Manistique and Benton Harbour locations, although trawl data were collected. For Benton Harbour we used the acoustic estimate from the closest adjacent transect, Saugatuck. For Manistique we used an acoustic estimate of fish abundance averaged over two inshore–offshore transects, one at Manistique and one at Charlevoix (Fig. 1), that was independently determined using the same acoustic technology as ours on the

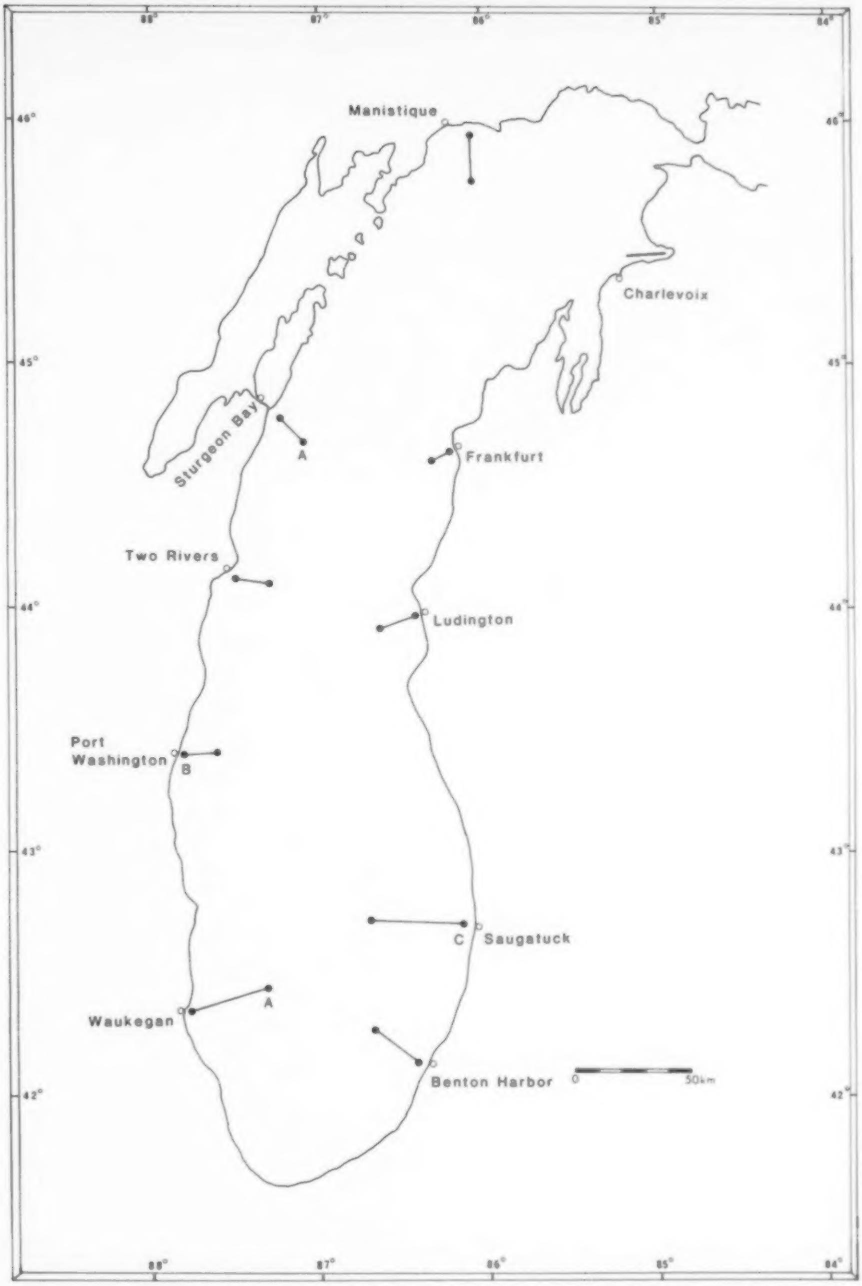
88°
87°
86°
85°
84°
46°
45°
44°
43°
42°
Manistique
Charlevoix
Sturgeon Bay
A
Frankfurt
Two Rivers
Ludington
Port Washington
B
C
Saugatuck
Waukegan
A
Benton Harbor
0
50km

FIG. 1. Lake Michigan showing transect locations. Solid circles designate location of plankton sampling stations (no such samples were taken at the Charlevoix transect). A = no summer phytoplankton sample taken, B = no summer phytoplankton or zooplankton sample taken, C = no spring zooplankton or phytoplankton sample taken.

same date (R. Argyle, U.S. Fish and Wildlife Service, 1451 Green Road, Ann Arbor, MI, pers. comm.).

Three species of fish, the alewife (*Alosa pseudoharengus*), rainbow smelt (*Osmerus mordax*), and bloater (*Coregonus hoyi*), that constitute the majority of pelagic planktivore biomass in Lake Michigan were considered in our study. Trawled fish were identified to species and measured for length, usually on freshly caught specimens, although some were determined from Formalin-preserved fish. Individual fish mass was determined from length – fresh weight regressions computed from 1985–87 Lake Huron specimens of these species (R. Argyle, U.S. Fish and Wildlife Service, 1451 Green Road, Ann Arbor, MI, unpubl. data). These equations were computed as direct, least-squares estimates of the nonlinear function

$$\text{weight} = \alpha \cdot \text{length}^{\beta}$$

as recommended by Zar (1968) and Pienaar and Thompson (1969). This procedure is necessary to minimize the likelihood of biased parameter (α and β) estimates that can result from least-squares linear regression analyses of logarithmically transformed allometric relationships.

The total density of fish determined acoustically was then apportioned among species and size classes according to trawl catch statistics. The trawl catch, however, underestimated the relative abundance of planktivorous fish that are smaller than about 40 g fresh mass, partly because these small fish pass through the trawl mesh, and partly because their vertical distribution may lie outside the bottom trawl's range. Thus before apportioning fish numbers amongst size classes, we back-calculated the number of smaller fish using a correction factor (Eck and Brown 1985) in combination with modelled size-frequency distributions for Lake Michigan planktivores (Hewett and Stewart 1989; DJS). For each transect these calculations produce estimates of mean planktivorous fish biomass (grams fresh biomass per square metre) which are consistent with our independent estimates based solely on acoustic data.

We estimated piscivorous fish biomass from energetics-based growth and population models (DJS) rather than from direct sampling. Modelled species were lake trout (*Salvelinus namaycush*), coho salmon (*Oncorhyncus kisutch*), and chinook salmon (*O. tshawytscha*). Wet weights of average individual salmonids ranged from 6 to 9000 g. We simulated lakewide populations and their size composition on dates of interest using known stocking data, observed size-at-age, and survivorship schedules for each species (Stewart et al. 1981, 1983). Estimates of lakewide biomass for the three modelled salmonids were extrapolated to include rainbow trout (*O. mykiss*) and brown trout (*Salmo trutta*). We assumed that contribution per individual stocked for the latter two species would equal that of the average individual for the three modelled species. Thus, it was necessary to increase modelling estimates for 1987 by 31.6% to approximate whole-lake salmonid biomass. Final estimates were expressed as grams fresh biomass per square metre.

The biomass size spectrum for a given transect at a particular time was generated as follows. The total size range of all organisms from picoplankton through to the largest salmon was transformed into a logarithmic scale of body mass (abscissa) so that the midpoint of one mass interval was double that of the previous one (i.e. 1, 2, 4, 8, 16,...g) (Sheldon and Parsons 1967). The total living biomass in each of these size intervals was then summed and plotted on the ordinate on a linear scale (Fig. 2). The plankton portion of the spectrum for a transect was the arithmetic mean of the inshore and offshore spectra (except when only a single sample was available, Fig. 1). No modification of planktivorous fish data was required, since these estimates were for the whole transect. Piscivorous fish data were not available for individual transects, since they were generated only for the total lake at each sampling period. Thus, piscivore data could be included only in the spring, summer, and seasonal mean spectra which were generated by simple arithmetic averaging of component size spectra.

Results and Discussion

Size Spectra

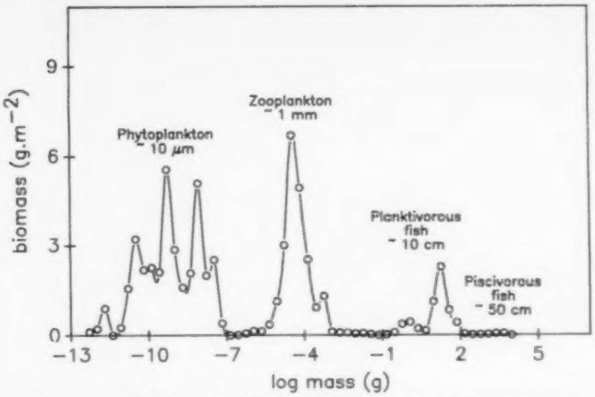

FIG. 2. Example of a Lake Michigan biomass–size distribution showing the component trophic groups and their typical maximum linear dimension.

Biomass spectra for individual transects comprise four major peaks corresponding to phytoplankton, zooplankton, planktivorous fish, and piscivorous fish (Fig. 2). Within each of these groups, minor peaks correspond to the various species or sizes of component organisms. During May, relatively high phytoplankton biomass reflects the typical spring algal bloom for Lake Michigan (Bowers et al. 1986) (Fig. 3). Concentrations tend to be higher in the warmer southern part of the lake, although high values at Manistique in the north may reflect nutrient input from productive Green Bay. Since the deep chlorophyll layer is not normally present during May in Lake Michigan (Fahnenstiel and Scavia 1987a), our integrated offshore phytoplankton samples provide reliable estimates of algal biomass. Total zooplankton biomass (Table 1) is slightly underestimated during the spring cruise, since our daytime plankton hauls did not generally capture *M. relicta* which migrates vertically at night. However, based on the one night haul in May and all (night) summer samples, this missing biomass would not exceed 2.5 g·m^{-2} and would more typically be about 0.3 ± 1.0 g·m^{-2}. We did not capture *P. hoyi* at any time. This benthic amphipod feeds primarily on sedimenting phytoplankton. It is high in storage lipid (Gardner et al. 1985) and is eaten by planktivorous fish, particularly the bloater (MacDonald et al. 1990). However, we were able to estimate the mean annual *P. hoyi* biomass from published data. Combined bloater, alewife, and smelt biomass shows modest geographic variation, with no obvious spatial pattern (Fig. 3). None of these planktivore species had spawned at the time of our spring cruise, so their biomass reflects only cohorts from previous years.

Summer mean phytoplankton biomass was 43% of the spring mean, and geographic variability was less (Table 1; Fig. 4). The deep chlorophyll layer is at its maximum in summer and occurs between 25 and 70 m (Fahnenstiel and Scavia 1987a). Therefore our integrated samples (36.6 m to surface) probably underestimated the phytoplankton biomass. Mean zooplankton biomass almost doubled from May (Table 1) with peak values tending to be higher on the western side of the Lake (Fig. 4). Our summer night estimates of 70 *Mysis*·m^{-2} in the offshore Saugatuck/Benton Harbour area agree well with estimates of 50 and 120 *Mysis*·m^{-2} in 1984 and 1985, respectively, at nearby Grand Haven (MacDonald et al. 1990). Mean planktivore biomass in August–September decreased to 5.7 g·m^{-2} from a mean May value of 6.4 g·m^{-2} (Table 1), again with no clear spatial pattern (Fig. 4).

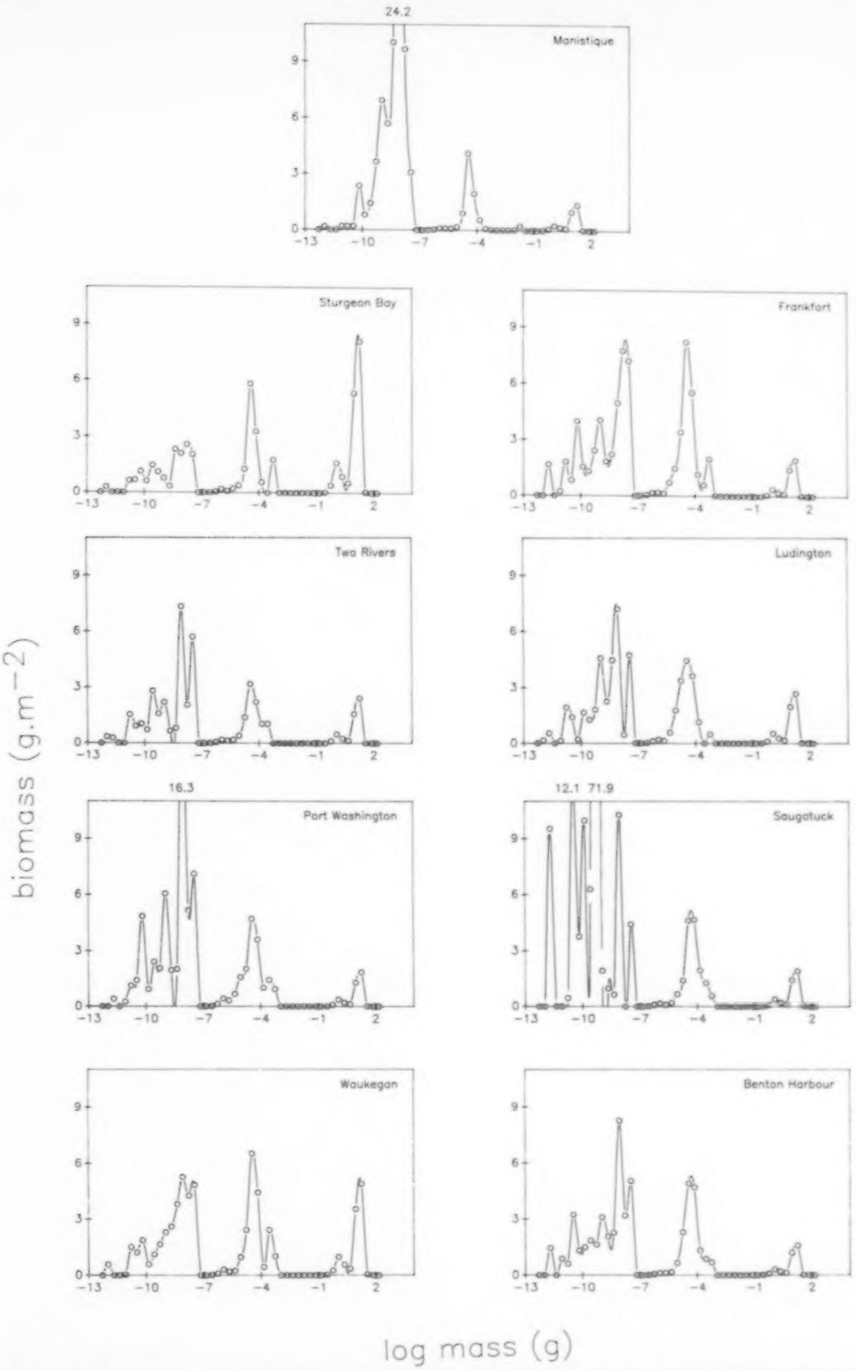

FIG. 3. Spring biomass–size distributions for all sampled transects. Spectra are arranged geographically with north at the top and west to the left (see Fig. 1).

Averaging the biomass spectra across locations within a time period reveals more clearly the temporal shifts in structure of the Lake Michigan food web and allows the addition of whole-lake modelling estimates of piscivore biomass (Fig. 5 and 6; Table 1). The spring phytoplankton bloom is clearly reflected in the greater area under this peak compared with its summer counterpart (Fig. 5 and 6). The presence of larger organisms, particularly diatoms such as *Melosira* and *Tabellaria* (Fahnenstiel and Scavia 1987b), is indicated by the right skew of the spring biomass peak (Fig. 5). By contrast, the summer

TABLE 1. Observed biomass for the trophic groups. Piscivore values are from a bioenergetic model estimate (see text). Spring and summer values are arithmetic means (with standard errors) of nine transects. Annual values are means of 18 transects, nine from each season.

	Biomass ($g \cdot m^{-2}$)		
	Spring (May 21–30)	Summer (Aug. 27 – Sept. 9)	Annual
Phytoplankton	48.8 (11.6)	21.2 (2.4)	35.0 (6.6)
Zooplankton	15.5 (1.5)	28.0 (3.6)	21.7 (2.4)[a] 31.0[b]
Planktivores	6.4 (1.5)	5.7 (0.9)	6.1 (0.9)
Piscivores	0.16[c]	0.22[c]	0.19 (0.03)
Total	70.8 (11.0)	55.1 (3.9)	72.3 (6.0)

[a] Excluding estimated *P. hoyi* biomass (see text).

[b] Including estimated *P. hoyi* biomass.

[c] Only one estimate available for the whole lake.

phytoplankton peak is lower and is located further to the left due to the predominance of smaller organisms (Fahnenstiel and Scavia 1987b). The increase in summer zooplankton biomass (Fig. 6; Table 1) is a manifestation of secondary production supported by the spring algal bloom. There is no indication of any change in overall zooplankton body size.

Summer planktivore biomass is slightly less than in the spring (Fig. 6; Table 1). Spring planktivore biomass includes two peaks, a larger one comprising fish aged 2 and older and a secondary peak of 1-year-old fish. These smaller fish grow during the summer to become incorporated into the larger of the two summer biomass peaks (Fig. 6). The secondary summer planktivore peak comprises larval and juvenile fish that were spawned shortly after our spring cruise ended on May 30.

Biomass of trout and salmon predicted from the bioenergetics model is less than 1 $g \cdot m^{-2}$ in both spring and summer (Fig. 5 and 6; Table 1). Size distributions of planktivores and piscivores overlap, but over 96% of the piscivore biomass occurs in the last five size classes which contain no planktivore biomass.

The overall mean spectrum (Fig. 7; Table 1) is a summary of the biomass–size structure of the complete Lake Michigan pelagic food web with spatial differences and temporal dynamics averaged out. Peak biomasses of phytoplankton and zooplankton are very similar at 5.6 and 6.7 $g \cdot m^{-2}$, respectively, although phytoplankton spans roughly 5.5 orders of magnitude of organism mass compared with only 3 orders for zooplankton (Fig. 7). Peak planktivore biomass is lower (2.3 $g \cdot m^{-2}$) and these fish span about 3 orders of magnitude of size (Fig. 7). Total piscivore biomass (primarily the last five size clases) of 0.19 $g \cdot m^{-2}$ (Table 1) appears as an almost undetectable rise at the extreme right of the size spectrum (Fig. 7).

Predictions

Particle-size models are equilibrium models based on the flow of mass and energy from smaller to larger organisms in pelagic food webs (Kerr 1974; Sheldon et al. 1977; Silvert and Platt 1980; Borgmann 1982). The temporal and spatial changes in biomass structure we report here for Lake Michigan indicate that this community is not in equilibrium at any given time or place. However, Chesson and Case (1986) and DeAngelis and Waterhouse (1987) have argued that equilibrium models can provide reasonable approximations of large and heterogeneous ecosystems because the transient dynamics of individual patches can be swamped by adjacent ones in a large landscape. On this basis we argue that our seasonal mean spectrum, which is averaged over a large spatial scale and a moderate temporal one, approximates the equilibrial condition of the Lake Michigan pelagic community for 1987 and can thus be used as a basis for making predictions about the expected structure of this ecosystem.

Biomass

Borgmann (1982, 1987) showed that in a pelagic ecosystem at equilibrium the biomass of organisms between sizes W_x and W_y (mass) is given by

$$(1) \quad B_{xy} = b(W_y^{n-\epsilon} - W_x^{n-\epsilon})$$

where ϵ is the particle-size conversion efficiency, a measure of the efficiency of biomass transfer scaled by relative sizes of predators and prey, n is the exponent in the allometric production to biomass equation

$$(2) \quad P/B = aW^{-n},$$

and b is a fitted constant. Since we do not have a basis for estimating n and ϵ from our data, we use instead published values derived from a variety of pelagic ecosystems (Borgmann 1982, 1987; Minns et al. 1987). ϵ ranges in value from 0.2 to 0.26, n from 0.16 to 0.24, and $n - \epsilon$ from -0.02 to -0.04. We have thus used $n = 0.2$, $\epsilon = 0.22$ to 0.24, and therefore $n - \epsilon = -0.02$ to -0.04 to model variation in parameter values that might reasonably be expected to be typical of Lake Michigan. Using our mean seasonal estimate of combined phytoplankton and zooplankton (including *P. hoyi*) biomass and the associated size range of these organisms, we determine the value of b for each value of $n - \epsilon$ using equation 1 and then compute the expected biomass for all size groups of organisms (Table 2).

Except for piscivorous fish, the concentration of biomass in the various size groups conforms closely to expectation (Table 2). Mean seasonal phytoplankton and zooplankton biomass are both within 1 standard error of the range predicted, and planktivorous fish biomass falls only slightly below. Total observed pelagic biomass on a mean annual basis is 72.3 ± 6.0 (SE) $g \cdot m^{-2}$ (Table 1) compared with a predicted range of 78.8–85.3 $g \cdot m^{-2}$. We did not expect the bioenergetic model estimate of trout and salmon biomass to conform to the particle-size model prediction because these stocked species do not reproduce naturally in the lake and hence are partially decoupled from normal food web dynamics. Current piscivore feeding behavior, particularly of salmon, could also account for their low biomass. A major decline in alewife stocks during the 1980's due to heavy salmon predation (Kitchell and Crowder 1986; Scavia et al. 1987) resulted in rapid increase of the bloater population due to reduced alewife predation (MacDonald et al. 1990) or competition (Crowder 1986). Bloater, which constitute 72% of the mean annual planktivore biomass in Lake Michigan, live in deeper, colder waters than alewifes and are largely unavailable as salmon forage. Alewifes and smelt make up 80–90% of salmonid diets even though they comprise under 30% of planktivore biomass. Our prediction of piscivore biomass should be interpreted as that which could be supported in Lake Michigan if the stocks reproduced naturally and if all planktivore species were potentially available as prey.

Although the concentration of biomass in various size groups conforms generally to theory, details of its distribution across

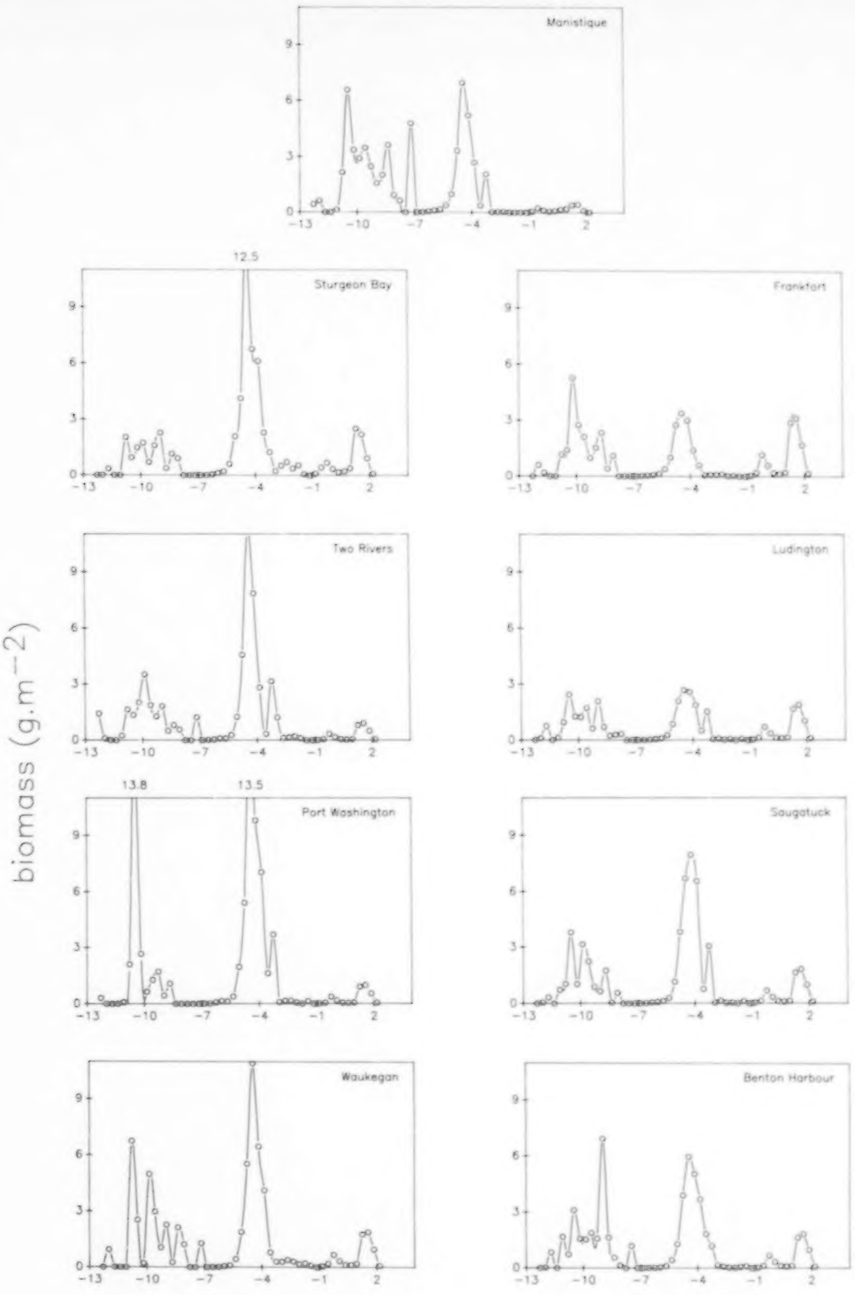

FIG. 4. Summer biomass–size distributions for all sampled transects. Arrangement as in Fig. 3.

organism size does not (Fig. 7). For steady-state systems, particle-size theory predicts a smooth, even decline in biomass from the smallest to largest organisms. Instead in Lake Michigan we observe a series of major biomass peaks separated by intervals of low or zero biomass. This is typical of what has previously been observed for freshwater plankton communities (Sprules et al. 1983) and appears to apply when fish biomass is included. The even distribution derives from the assumption that values of size-dependent turnover rate, n (equation 2), and biomass conversion efficiency, ϵ (equation 1), are constant throughout the food web. To the degree that values of these parameters change from one trophic group to another, the distribution of biomass will become less uniform. Departures from steady state caused by fluctuations in, for example, nutrient

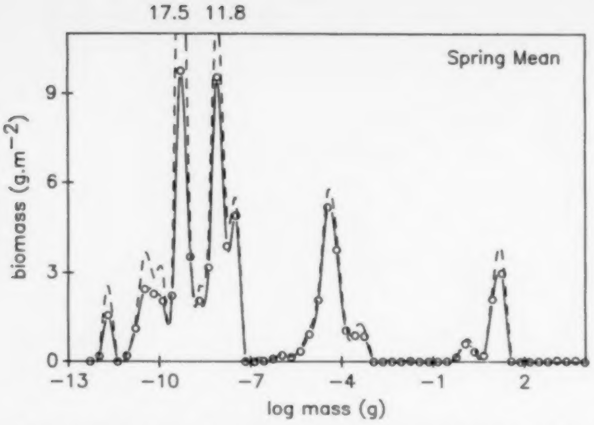

FIG. 5. Mean biomass–size distributions for spring. Broken lines designate 1 standard error above the mean.

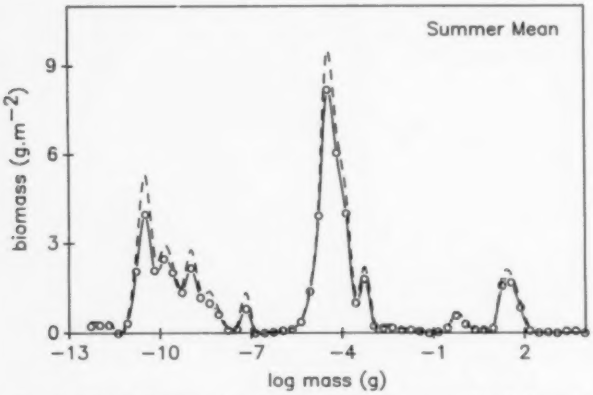

FIG. 6. Mean biomass–size distribution for summer. Symbols as in Fig. 5.

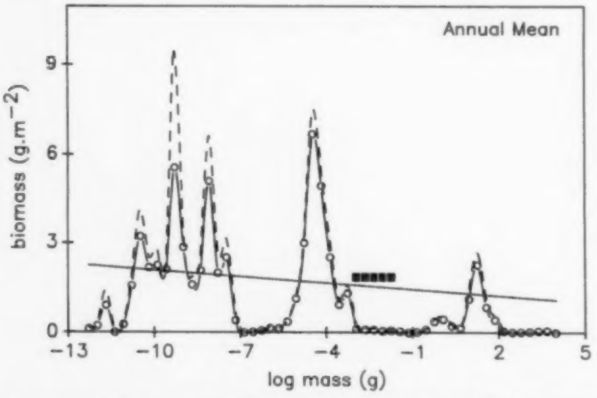

FIG. 7. Mean biomass–size distribution for the year. Solid squares represent estimated *P. hoyi* biomass (see text) and dash-dotted line is particle-size model prediction of biomass–size distribution corresponding to $n - \epsilon = -0.02$ (see text). Other symbols as in Fig. 5.

loading, predator year-class strength, or climate would have similar effects. Although detailed predictions of biomass for restricted size groups would thus appear not to be warranted until particle-size models are modified appropriately, predictions of general system structure for major size groups appear reasonable.

Production

It is also possible to predict potential production of any size group of organisms in the food web if actual production and body size are known for a reference group (Sheldon et al. 1977; Borgmann 1987). The only estimates of production available for Lake Michigan are for algal production during 1983 and 1984 (Fahnenstiel and Scavia 1987b). Minimal annual variation in primary production (G. L. Fahnenstiel, Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration, 2205 Commonwealth Blvd., Ann Arbor, MI, pers. comm.) suggests that the 1983–84 estimate can reasonably be used to estimate particle-size model parameters for 1987. Borgmann (1987) showed that production of organisms between sizes W_x and W_y is given by

$$(3)\quad P_{xy} = c(W_y^{-\epsilon} - W_x^{-\epsilon})$$

where ϵ is the particle-size conversion efficiency and c is a fitted constant. Once again we do not have the data to compute an estimate of ϵ for the Lake Michigan food web, but have chosen the values 0.22 and 0.24 to represent the limits within which it might reasonably be expected to fall (see above). On the basis of the 1983 and 1984 Lake Michigan data, Scavia (1988) estimated mean annual primary production to be 232 g C·m^{-2}·yr^{-1}. Using a C to biovolume ratio of 0.15 (Laws et al. 1984), we calculate mean annual primary production to be 1547 g·m^{-2}·yr^{-1}. Substituting this estimate and the size range of algae we sampled into equation 3, we estimate the potential annual production for the various size groups in the Lake Michigan food web (Table 3).

To our knowledge, very few estimates of production are available for Lake Michigan, and we know of none for herbivorous zooplankton or planktivorous fish. Sell (1982), however, estimated production of *M. relicta* and *P. hoyi* in the lake from various years between 1954 and 1979 using size–frequency techniques. From his summary, we have derived two estimates of the combined production of these two species (Table 3). The first is a summation of Sell's (1982) minimum and maximum values for the two species using a dry to fresh weight ratio of 0.15 to convert from Sell's dry weight calculations. The second is based on mean production to biomass ratios of 0.78 and 2.8, respectively, for *P. hoyi* and *M. relicta* calculated from Sell (1982) and applied to our 1987 estimates of mean annual biomasses of 9.3 and 1.16 g·m^{-2}, respectively, for the two species. There is agreement between predicted and estimated production for *P. hoyi* and *M. relicta*, although the particle-size model estimates may be low (Table 3).

Our only other independent production estimate is the one derived from the bioenergetic model for trout and salmon combined (DJS) (Table 3). This falls in the range predicted by the particle-size model, indicating good agreement between these two approaches.

Conclusion

Because it is costly and time-consuming to document patterns of production and biomass for whole food webs based on studies of component populations, the use of whole system models of the sort we present here should be considered. We argue that mean seasonal biomass in the Lake Michigan pelagic

TABLE 2. Observed and predicted biomass for various size groups. Predictions are from Borgmann's (1982) particle-size model; observations are from Lake Michigan seasonal mean (± standard error) size spectrum, except for piscivores which is derived from the bioenergetics model.

			Biomass ($g \cdot m^{-2}$)		
	Mass (g)		Predicted $n - \epsilon$		
	Minimum	Maximum	−0.02	−0.04	Observed
Phytoplankton	3.7×10^{-13}	9.7×10^{-8}	36.3	40.4	35.0 ± 6.6
Zooplankton	9.7×10^{-8}	0.05	29.7	25.6	31.0 ± 2.4[a]
Planktivores	0.1	208	13.7	9.4	6.1 ± 0.9
Piscivores[b]	208	6667	5.6	3.4	0.19 ± 0.03
Total	3.7×10^{-13}	6667	85.3	78.8	72.3 ± 6.0

[a] Includes 9.3 $g \cdot m^{-2}$ *Pontoporeia* biomass estimated from the literature (see text).

[b] This size range includes over 96% of piscivore biomass with the remainder falling in the previous few planktivore size intervals.

TABLE 3. Potential annual production predicted from the particle-size model for the various trophic groups. Observed phytoplankton production from Scavia (1988); observed piscivore production is from the bioenergetics model.

			Production ($g \cdot m^{-2} \cdot yr^{-1}$)		
	Mass (g)		Predicted ϵ		
	Minimum	Maximum	0.22	0.24	Observed
Phytoplankton	3.7×10^{-13}	9.7×10^{-8}	—	—	1547
Herbivorous zooplankton	9.7×10^{-8}	8×10^{-4}	91.6	72.1	—
Mysis, Pontoporeia	8×10^{-4}	0.1	9.6	6.5	10.6[a] 7–61.3[b]
Planktivores	0.1	208	4.1	2.5	—
Piscivores[c]	208	6667	0.50	0.27	0.27

[a] Estimated using *P/B* ratios from Sell (1982) and our mean annual biomasses (see text).

[b] Range of values calculated by Sell (1982) (see text).

[c] Piscivore biomass as in Table 2.

food web is a good approximation to the steady-state structure of this system which can then be described by an equilibrium particle-size model. We choose ranges of parameter values that are known to reasonably approximate the structure of pelagic food webs in nature and consider our subsequent predictions of biomass and potential production to be reasonably accurate. The spatial scale of our sampling program was extensive, and the two major periods of spring mixing and summer thermal stability were included.

Our results suggest that the particle-size approach to analyses of food web structure and dynamics in large ecosystems such as Lake Michigan is promising. Particle-size estimates of mean annual biomass of phytoplankton, zooplankton, and planktivorous fish conformed closely to observed values (Table 2). There was also correspondence between predicted and calculated values of macroinvertebrate and piscivorous fish production (Table 3). The model's success even though parameter values were derived from other aquatic ecosystems rather than estimated directly from Lake Michigan data suggests that particle-size precepts may apply quite generally. They have been successfully applied in an extensive series of production analyses in Lake Ontario (Borgmann et al. 1984; Minns et al. 1987) and to estimates of sustainable anchoveta production (Sheldon et al. 1977). Although predictions for organisms in major size intervals appear to be rigorous, fine-scale detail of biomass distribution does not conform to theory, at least for freshwaters (Fig. 7). Some slight evidence suggests that biomass–size distributions in the ocean are smoother and more uniform as predicted by theory (Sheldon et al. 1972; Hargrave et al. 1985; Rodriguez and Mullin 1986). In our view, the great utility of the particle-size model is its comparative simplicity that leads to predictions about the general structure and dynamics of aquatic ecosystems; attempts to increase model complexity to account for finer scale detail would be counterproductive.

The one major discrepancy in particle-size prediction was with piscivorous fish biomass, although this was expected because these species are stocked. Nevertheless the model provides useful insight into the current Lake Michigan pelagic community. We have argued that low piscivore biomass is partly due to the unavailability of bloater prey, the major constituent of planktivore biomass. Evidence suggests that salmon feed primarily on the much less abundant alewife and smelt (DJS). Despite their low biomass relative to model predictions (Table 2), piscivores nevertheless achieve production levels consistent with the model (Table 3). This is almost certainly due to the fact that, for their size, salmon have unusually high production to biomass ratios (DJS). This is especially true for chinook salmon that make up over half the salmonid production in Lake Michigan and have a P/B ratio of 1.6, compared with only 0.6 for lake trout.

It would appear, then, that much of the energy flowing from the base of the food web in Lake Michigan is unavailable to piscivores because they cannot utilize the abundant bloater. Nevertheless, salmonid production in the lake conforms to the particle-size model prediction (Table 3, $\epsilon = 0.24$) which implicitly assumes all planktivore production flows to piscivores. This discrepancy is due to the high efficiency of energy conversion by some of the piscivores and suggests that a more reasonable estimate of their potential production should be based on the lower value of $\epsilon = 0.22$ (the absolute value of ϵ varies inversely with conversion efficiency). Thus if all planktivore production were used, piscivore production could potentially reach $0.5\ \mathrm{g{\cdot}m^{-2}{\cdot}yr^{-1}}$ (Table 3), or double current values. Food web structure in Lake Michigan thus constrains salmonid production. Further stocking of salmon and trout into the lake would be ill-advised unless there were concurrent management practices aimed at establishing higher stocks of planktivores that can be efficiently utilized by salmonids.

Acknowledgements

We are grateful for financial support of this study from the Natural Sciences and Engineering Research Council of Canada, the Donner Canadian Foundation, the Wisconsin Department of Natural Resources, and the U.S. Federal Aid in Sport Fish Restoration Act under Project F-95-P. The support of captains and crew of the R/V *Neeskay*, R/V *Steelhead*, and R/V *Cisco* is much appreciated.

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Functional Interaction of Phytoplankton and Zooplankton along the Trophic Gradient in Green Bay, Lake Michigan[1]

Paul E. Sager

Department of Natural and Applied Sciences, University of Wisconsin, Green Bay, WI 54311, USA

and Sumner Richman

Department of Biology, Lawrence University, Appleton, WI 54912, USA

Sager, P. E., and S. Richman. 1991. Functional interaction of phytoplankton and zooplankton along the trophic gradient in Green Bay, Lake Michigan. Can. J. Fish. Aquat. Sci. 48: 116–122.

The functional interaction of phytoplankton and zooplankton, expressed in terms of the numerical difference between phytoplankton growth rates per day (in situ ^{14}C method) and zooplankton grazing rates per day (in situ feeding experiments), was studied along the trophic gradient in Green Bay, Lake Michigan. Growth–grazing differences increased with trophic conditions, averaging 0.08 for the water column in the meso-oligotrophic northern bay and 0.56 in the eutrophic southern bay for the summers of 1986, 1987, and 1988. Eutrophic conditions produced dominance of growth by large-size cyanobacteria and low grazing rates by microcrustaceans. Small and occasionally negative growth–grazing differences in the meso-oligotrophic region were associated with dominance of larger cladocerans and calanoid copepods and small algal species. Phytoplankton growth rates in the northern bay averaged about 28% those of the eutrophic region. A unimodal phytoplankton growth response to increased grazing was observed in the northern bay, suggesting variation in positive (growth stimulating) and negative (grazing losses) effects of zooplankton on the phytoplankton.

L'interaction fonctionnelle du phytoplancton et du zooplancton, exprimée en termes de différence numérique entre les taux de croissance du phytoplancton par jour (méthode ^{14}C in situ) et les taux d'alimentation du zooplancton par jour (essais d'alimentation in situ), a été étudiée pour le gradient trophique de Green Bay, au lac Michigan. Les différences croissance–alimentation s'accentuaient avec les conditions trophiques, atteignant en moyenne 0,08 pour la colonne d'eau dans la baie méso-oligotrophique nord et 0,56 dans la baie eutrophique sud, pendant l'été 1986, 1987 et 1988. Les conditions eutrophiques sont à l'origine d'une prédominance de la croissance par les grosses cyanobactéries et de faibles taux d'alimentation par les microcrustacés. Les différences peu prononcées et, à l'occasion, négatives entre la croissance et l'alimentation dans la région méso-oligotrophique étaient associées à la prédominance de copépodes calanoïdes et de cladocères plus gros, ainsi qu'aux petites espèces d'algues. Les taux de croissance du phytoplancton dans la baie nord correspondaient en moyenne à 28 % environ de ceux observés dans la zone eutrophique. Une réaction de croissance unimodale du phytoplancton à l'accroissement de l'alimentation a été observée dans la baie nord, ce qui porte à croire à une variation des effets positifs (stimulation de la croissance) et négatifs (baisses dans l'alimentation) du zooplancton par rapport au phytoplancton.

Received February 26, 1990
Accepted August 30, 1990
(JA708)

Reçu le 26 février 1990
Accepté le 30 août 1990

The relative importance of nutrient status and food web effects in phytoplankton–zooplankton interactions has received much attention recently, with particular interest placed on phytoplankton responses and water clarity (Shapiro and Wright 1984; Kitchell et al. 1987; Scavia et al. 1986; Mazumder et al. 1990). Many of these studies have dealt with examination of static variables, e.g. biomass, standing crop. Other studies suggest that the relative importance of biotic and abiotic effects is better understood in terms of dynamic processes such as feeding, growth, and assimilation (Scavia and Fahnenstiel 1987; Sager and Richman 1990; Simons and Lam 1980). Still others have noted significant influences of trophic condition of the water body (McQueen et al. 1986) and of the food web complexity or number of trophic levels (Persson et al. 1988) on the dynamic processes.

The latter studies call for a comparative focus on functional aspects or rate processes to elucidate the nature of trophic level interactions in lake ecosystems. Empirical evidence from concurrent measurements of growth and grazing processes is seldom available for a given lake and rarely for a water body with variable trophic conditions. The purpose of this study is to examine the interaction between phytoplankton growth and zooplankton grazing along a trophic gradient in a single water body, Green Bay, Lake Michigan.

Green Bay has unique freshwater estuarine features with hypertrophic conditions characterizing the shallow (z = 2–3 m) southern end, largely due to nutrient inputs from the major tributary, the Fox River. Meso-oligotrophic conditions characterize the deeper (z = 30 m) northern area (Conley 1983; Sager et al. 1984; Richman et al. 1984; Auer et al. 1986). The bay is 190 km long, 37 km in mean width, and about 4000 km^2 in area.

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[1] This paper is one of a series originating from a special session of the 32nd Conference on Great Lakes Research (Madison, WI, May 30 to June 2, 1989) entitled "Population Dynamics of Large Lake Ecosystems: Patterns, Processes, and Interlake Comparisons."

Methods and Procedures

Field sampling was done in summer on five cruises in 1986 and four each in 1987 and 1988 at 10- to 14-d intervals beginning in mid-June. Samples were collected with a Van Dorn type sampler from six depths throughout the water column at three stations (Fig. 1). At the lower bay station (LB), water depth was 6 m; samples were taken at 0, 1, 2, 3, 5, and 6 m. At the midbay station (MB), water depth was 25 m; samples were taken at 0, 3, 6, 10, 16, and 24 m. At the upper bay station (UB), water depth was 29 m; samples were taken at 0, 3, 6, 10, 16, and 26 m. Thermal stratification had developed at MB and UB by the time of the first cruises in June. The water column at LB did not stratify except in August 1988 due to intrusion of cold, hypolimnetic water from the middle bay.

Productivity was determined in situ by the ^{14}C method from incubation periods averaging 4 h, generally between 1000 and 1400. One millilitre (296 kBq) of [^{14}C]NaHCO$_3$ was added to each of two light bottles (260 mL Pyrex) and one "DCMU" bottle for each of the six depths. The DCMU bottle received 0.5 mL of 0.005 M (DCMU (Diuron), 3-(3,4-dichlorophenyl)-1,1-dimethyl urea, as a photosynthetic inhibitor (Legendre et al. 1983). Productivity samples were filtered on Whatman GF/F filters (mean pore size 0.7 μm), rinsed with 0.5 N HCl, and counted in a Beckman 1801 liquid scintilation system. Integrated, daily solar radiation (PAR) was determined from continuous pyrrheliometer recordings at the University of Wisconsin-Green Bay campus on the south shore of Green Bay. Estimates of daily volumetric production (milligrams C per cubic metre per day) and integral production (milligrams C per square metre per day) were extrapolated from the 4-h incubation measurements using the ratio of daily PAR to that of the incubation period (Wetzel and Likens 1979). Samples for chlorophyll *a* were filtered on Whatman GF/F filters and analyzed

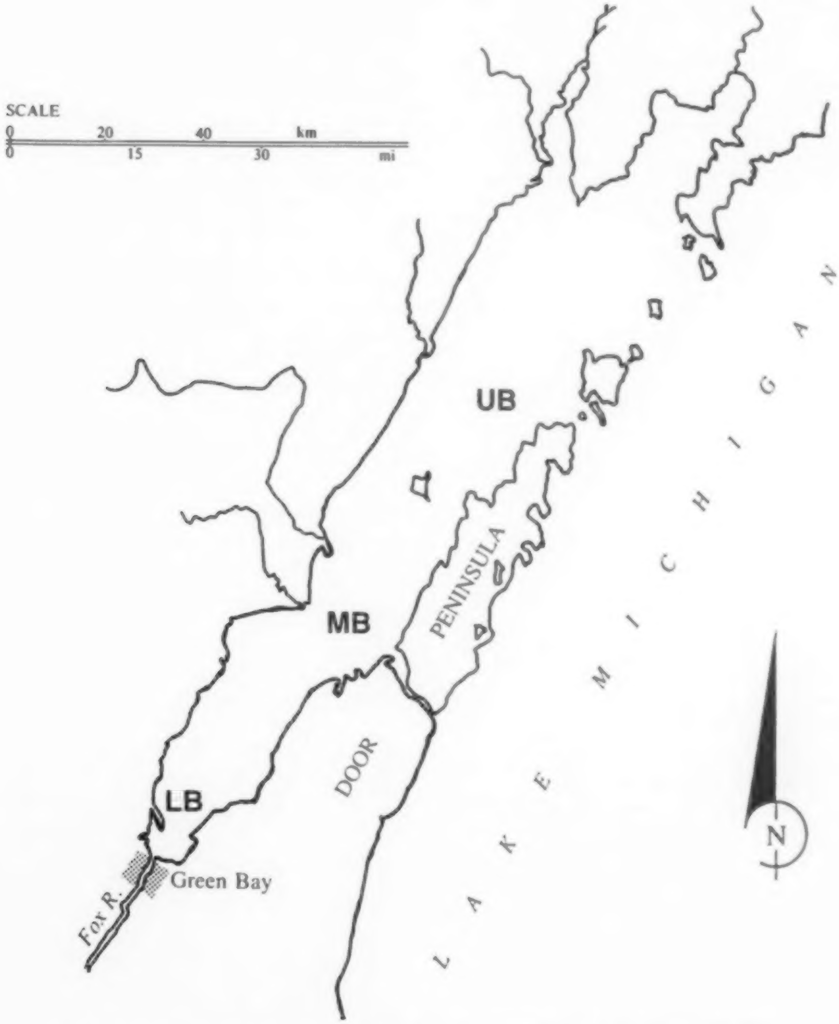

FIG. 1. Location of sampling stations LB, MB, and UB, Green Bay, Lake Michigan.

according to Lorenzen (1967). From concurrent sampling, phytoplankton biovolume measurements were made on a model B Coulter Counter interfaced to a Commodore PET 2001 microcomputer. Results from counts with aperture tubes of 140 and 200 μm were combined to yield a size range of 30 to 300 000 μm^3 corresponding to 4–90 μm in equivalent spherical diameter.

Biovolume (*B*) measurements (parts per million by volume) were converted to particulate carbon (PC) according to the relationship taken from Sheldon and Parsons (1967):

$$\mathrm{PC}\ (\mathrm{mg\ C{\cdot}m^{-3}} = [(B + 3.56)/19.0].$$

As the biolvolume measurements do not distinguish detrital particles from living algae, a correction for detrital C must be made in the PC estimates to yield algal C biomass. Fahnenstiel and Scavia (1987) reported algal C to chlorophyll ratios (based on cell counts and subsequent C conversions) to average 38.6 for the epilimnetic community in Lake Michigan during summer stratification. At UB, the most comparable of the Green Bay stations with Lake Michigan, summer chlorophyll levels ranged from 0.84 to 6.68 $\mu g{\cdot}L^{-1}$, averaging 3.23 for the three years. Using the C to chlorophyll ratio of Fahnenstiel and Scavia, the corresponding average algal C biomass estimate at UB would be about 120 $mg\ C{\cdot}m^{-3}$ or 40% less than the corresponding, uncorrected PC estimate (205 $mg\ C{\cdot}m^{-3}$). This factor of 0.6 is thus used to correct for detrital C in PC estimates at UB.

Because average chlorophyll to biovolume ratios for the three stations are variable, ranging from 4.3 to 6.1 to 6.8 for UB, MB, and LB, respectively, a single correction for detrital C cannot be used at all stations. Peterson (1978) reported a range of 22–84% of particulate organic carbon (POC) for the algal C fraction in Cayuga Lake. Harris (1986) also noted the detrital fraction to be variable and less important as algal biomass increases, becoming almost insignificant at high algal biomasses (200–300 $mg\ Chl{\cdot}m^{-3}$).

To derive a correction factor for MB and LB, we use the above chlorophyll to biovolume ratios as indicators of the change in proportion of detritus in the PC estimates relative to the correction for UB data. Thus for MB the chlorophyll to biovolume ratio (6.1) is 1.42 times greater than at UB (4.3) and the correction for the algal C fraction would be 0.85 or 1.42 times the corresponding correction (0.60) for UB. Similarly for LB data the chlorophyll to biovolume ratio (6.8) is 1.58 times that for UB (4.3) and the correction for the algal C fraction becomes 0.95 (1.58 × 0.60).

Zooplankton feeding experiments were done during daytime in situ at the same depths as above according to Lampert and Taylor (1985) with a 3-L feeding chamber modified slightly from Haney (1971) (see also Richman et al. 1990). Three millilitres of ^{14}C-labelled algae (either *Chlamydomonas, Scenedesmus*, or *Ankistrodesmus*, maximum dimensions of 14, 25, and 47 μm, respectively) suspensions was added to the chamber resulting in increases over ambient concentrations of approximately 150 $cells{\cdot}mL^{-1}$ for each feeding experiment. After incubation for approximately 7 min the zooplankton were filtered through an 80-μm plankton mesh fitted in a Plexiglas cylinder and washed in two successive baths of soda water to anaesthetize them and remove any uningested radioactive algae. Zooplankton were collected on 0.45-μm Millipore filters and placed in scintillation vials. Subsamples (2 mL) from the chamber were filtered (0.45 μm Millipore filters) and used to determine phytoplankton and dissolved radioactivities. A Beckman 230 liquid scintillation system was used to measure radioactivities. Equations from Lampert and Taylor (1985) were used to calculate the community grazing rate (millilitres per litre per hour). Averages of measurements based on the three food species of algae are used in the discussion to follow, since there were no obvious differences in feeding rates for the three indicator species.

TABLE 1. Water column mean biovolume measurements (ppm ± SD) and ratios (Z/P) for zooplankton and phytoplankton from summers of 1987 and 1988 at LB, MB, and UB stations (see Fig. 1) on Green Bay (after Richman and Sager 1990). Differences among means for each of phytoplankton, zooplankton, and Z/P are significant (single-factor ANOVA, $p < 0.01$). Station comparisons (Tukey test) of phytoplankton are significant ($p < 0.01$) for LB with MB and UB, of zooplankton ($p < 0.01$) for UB with LB and MB, and of Z/P for LB with UB ($p < 0.01$) and MB ($p < 0.05$).

	n	LB	MB	UB
Phytoplankton	46	3.45 ± 2.18	1.01 ± 0.46	0.70 ± 0.42
Zooplankton	46	2.82 ± 1.67	2.19 ± 1.78	1.24 ± 0.96
Z/P	46	1.14 ± 1.16	2.74 ± 2.83	3.44 ± 4.64

TABLE 2. Water column means (± SD) of individual ($mL{\cdot}animal^{-1}{\cdot}d^{-1}$) and community ($mL{\cdot}L^{-1}{\cdot}d^{-1}$) zooplankton grazing rates from summers of 1986, 1987, and 1988 at LB, MB, and UB stations (Fig. 1) on Green Bay (after Sager and Richman 1990). Differences among means are significant (single-factor ANOVA, $p < 0.05$) only for individual grazing rates. Station comparisons (Tukey test) for individual grazing rates are significant ($p < 0.05$) only for LB with UB.

	n	LB	MB	UB
Individual	11	0.96 ± 0.72	2.88 ± 2.88	7.20 ± 10.6
Community	11	111.8 ± 51.4	95.0 ± 32.9	109.2 ± 81.8

Depth profiles of light were made with a LiCor radiometer (Li-1858) and underwater sensor (Li-192SB). Depth of the photic zone was calculated as the depth of 0.5% of surface light ($z_{0.5}$). Water temperatures were measured with a Kahl thermometer.

Statistical analysis of data involved ANOVA followed by Tukey tests to determine significance of comparisons of means between stations.

Results and Discussion

In a previous report from this study (Richman and Sager 1990), we described significant differences in total biovolume of phytoplankton and zooplankton along the trophic gradient in Green Bay (Table 1). The biovolume ratios in these data suggest that the interaction between these two communities and especially the grazing effectiveness of the zooplankton change with trophic conditions. Examination of average grazing rates along the trophic gradient substantiates this suggestion in part (Table 2). Community grazing rates are similar at the three sampling stations. Sterner (1989) also noted that there is no evidence that community grazing rates systematically vary along an oligotrophic–eutrophic gradient. Individual grazing rates, however, significantly increase in the meso-oligotrophic region. Because of lower abundance of zooplankton, community grazing rates at UB do not follow the trend of increased, individual rates in this meso-oligotrophic region of the bay.

Phytoplankton Growth and Loss Processes

From the similarity in community grazing rates observed for the three regions of Green Bay (Table 2), we might conclude that there is little effect of trophic conditions on phytoplankton losses due to grazing. Growth as well as loss processes such as grazing influence the state of the phytoplankton biomass. Grazing has been long cited as a potential control on phytoplankton abundance (Riley 1946; Gliwicz 1969; Haney 1973; Lampert and Taylor 1985). Sedimentation or sinking of algal cells may also be a significant loss process (Scavia and Fahnenstiel 1987). Growth represents a gain in biomass and may, depending on its magnitude, counter biomass losses due to grazing and other processes. The outcome of the interaction between recent gains and losses determines the abundance of phytoplankton at any time (Forsberg 1985).

Substantial variations in productivity of the phytoplankton can be observed among the three stations sampled in this study (Fig. 2). Maximum rates of volumetric productivity at the depth of optimum light conditions in the water column (p_{max}) may vary by more than 20-fold along the trophic gradient. These variations have relevance to the impact of grazing along the trophic gradient.

To examine this interaction, expressions of phytoplankton growth and zooplankton grazing with common units are needed. We have estimated phytoplankton growth rates from data on ^{14}C productivity and corresponding estimates of algal carbon biomass. Daily volumetric productivity as a fraction of algal biomass has been used as a measure of specific growth rate (Parker et al. 1977; Peterson 1978; Harris 1986; Fahnenstiel and Scavia 1987) on the assumption that daily growth is proportional to photosynthetic production of cell cover a 24-h period. While this proportionality is generally accepted (Malone 1982), it is recognized that photosynthesis rates are more variable than cell division rates over short time periods. Hence, long ^{14}C incubation times (24 h) are sometimes favored for making estimates of daily production or growth rates (Sournia 1974; Eppley and Sharp 1975; Fahnenstiel and Scavia 1987). Because of sampling logistics and a concern for containment effects (Wetzel and Likens 1979; Reynolds 1984) in the fertile waters of Green Bay, we have used short incubation times (4 h) and have made estimates of phytoplankton growth rates (per day) on this basis. Community grazing rates (millilitres per litre per day) in Table 2 divided by 10^3 produce a daily rate yielding common units for the growth and grazing expressions.

Growth–Grazing Interactions

As a means of examining the variations in functional interaction between phytoplankton growth and zooplankton grazing, numerical differences between the two expressions can be compared from data of the three stations along the trophic gradient:

$$F = [\text{growth}\cdot d^{-1} - \text{grazing}\cdot d^{-1}].$$

Data on grazing, productivity, and phytoplankton biomass are available for each of six depths sampled in the water column, permiting calculation of the difference (F) for each depth. F will vary with depth, a major influence being the light-dependent variation in productivity and growth of the phytoplankton. Grazing rates, phytoplankton biomass, and zooplankton biomass are also not uniformly distributed with depth but are much less variable than rates of productivity (Richman et al. 1990). Analysis of depth-related factors affecting F is beyond the intent of this study; hence, we have calculated F from mean water column values of growth and grazing.

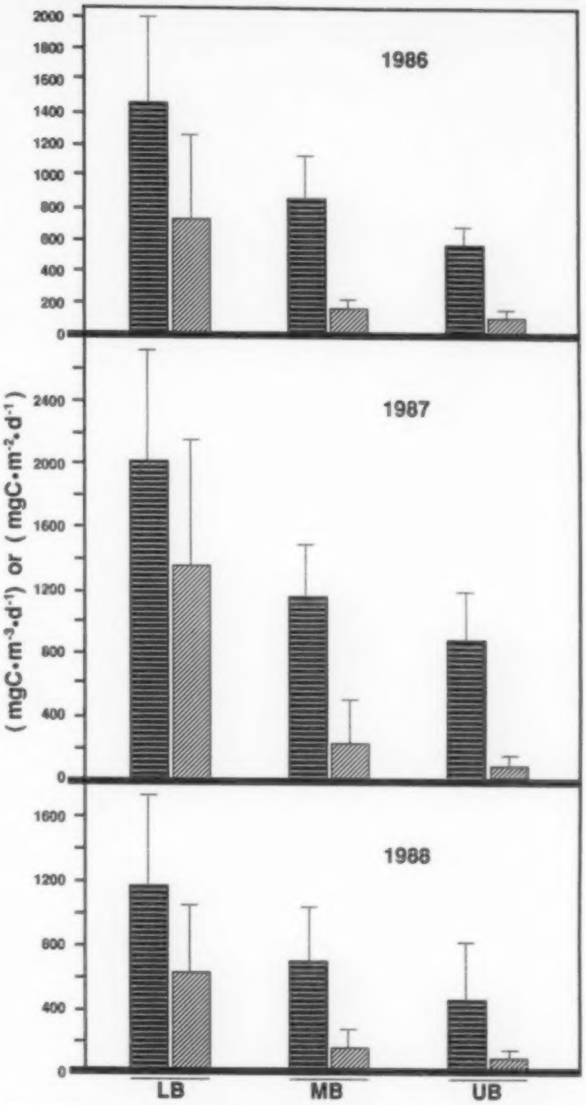

FIG. 2. Summer means (with standard error bars) of integral production (mg C·m^{-2}·d^{-1}; cross-hatched) and p_{max}, volumetric production (mg C·m^{-3}·d^{-1}; diagonal-hatched) at LB, MB, and UB stations on Green Bay.

Depth integrals of daily production and areal phytoplankton biomass divided by the depth (metres) of the photic zone ($z_{0.5}$) yield mean volumetric values of each for calculating a water column growth rate at each station. Significant differences as expected in this growth term are found between LB and the other stations along the trophic gradient (Table 3).

The corresponding water column term for grazing rate is calculated as the average of values for the sampling depths at a station. From the 3-yr study, 33 F values, 11 from each station, are available for analysis.

Distribution of F

Values of F for all stations and sampling dates vary from -0.11 to 1.03, indicating a wide range in relative importance

TABLE 3. Water column means (± SD) of ^{14}C-based, specific growth rates (d^{-1}) for phytoplankton of Green Bay at stations LB, MB, and UB (Fig. 1) from summers of 1986, 1987, and 1988. Differences among means are significant (single-factor ANOVA, $p < 0.01$). Station comparisons (Tukey test) are significant ($p < 0.01$) only for LB in relation to MB and to UB.

	LB	MB	UB
Mean	0.68 ± 0.28	0.19 ± 0.08	0.19 ± 0.09
Range	0.34–1.25	0.08–0.25	0.04–0.34
n	13	12	12

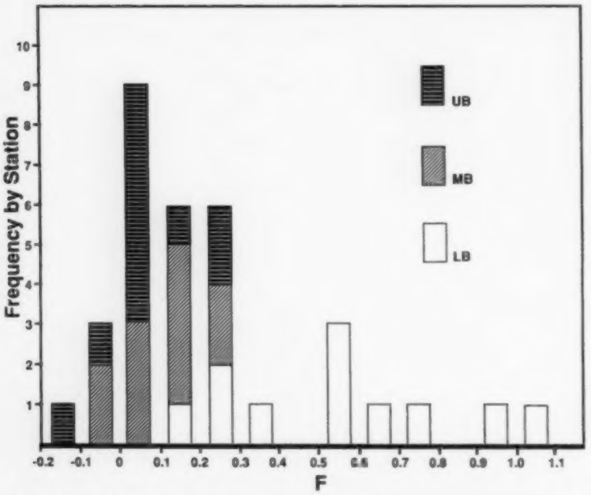

FIG. 3. Distribution of numerical differences (*F*) between water column means of phytoplankton growth rates (d^{-1}) and zooplankton grazing rates (d^{-1}) among sampling stations LB, MB, and UB on Green Bay from summers of 1986, 1987, and 1988.

TABLE 4. Water column means (± SD) of *F*, the numerical difference between phytoplankton growth rates (d^{-1}) and zooplankton grazing reates (d^{-1}), at LB, MB, and UB stations (Fig. 1) on Green Bay from summers of 1986, 1987, and 1988. Differences among means are significant (single-factor ANOVA, $p < 0.01$). Station comparisons (Tukey test) are significant ($p < 0.01$) only for LB in relation to MB and to UB.

	LB	MB	UB
Mean	0.56 ± 0.29	0.09 ± 0.11	0.08 ± 0.11
Range	0.11–1.03	−0.01–0.25	−0.11–0.27
n	11	11	11

of grazing and growth processes along the trophic gradient in Green Bay. While some overlap in values occurs among the three stations, the greatest differences in growth and grazing rates are associated with the hypertrophic LB station (Fig. 3). For the meso-oligotrophic MB and UB sites, most values are less than 0.25, including four that are negative. Mean values, based on the data at each station for all three years, show significant differences between LB and the other two stations (Table 4). The functional interaction of phytoplankton and zooplankton expressed in growth–grazing rate differences thus varies significantly along the trophic gradient in Green Bay.

Growth Dominance at LB

At LB, with values of *F* being generally high, grazing appears not to be a major loss process for the phytoplankton community as a whole. High levels of productivity and growth in the eutrophic waters compensate for apparently low grazing losses. The low grazing impact can be related to the composition and size distribution of both the phytoplankton and zooplankton. Richman and Sager (1990) noted a decrease in mean size of phytoplankton (cell volume converted to spherical equivalent diameter) from 54.8 to 28.6 to 22.4 μm at LB, MB, and UB stations, respectively. Sager et al. (1988) found the proportion of small size (<10 μm) fractions of the phytoplankton to decrease from more than 50% in northern Green Bay to less than 10% in the southern Bay. The summer genera of phytoplankton at the eutrophic LB station, while including a mixture of diatoms, greens, and blue-greens, were dominated by *Anabaena* and *Microcystis*, which provide low-quality food for herbivorous zooplankton (Gliwicz 1977; Richman and Dodson 1983; Webster and Peters 1978).

The summer zooplankton at LB were dominated by small crustaceans (*Bosmina*, *Chydorus*, and *Cyclops*). The low grazing effectiveness of these small zooplankters (Bogdan and Gilbert 1982; Demott and Kerfoot 1982) and the low food quality of the phytoplankton suggests that a substantial fraction of the primary production passes not through the pelagic food chain but to the benthic–detrital food chain.

Grazing Dominance at MB and UB

In comparison with LB, values of *F* at UB and MB are significantly lower and occasionally negative (Fig. 3), suggesting greater zooplankton control of the phytoplankton. Average estimates of phytoplankton growth rates are about 28% those at LB (Table 3). Daily gains in phytoplankton biomass from these low growth rates are at times probably offset by grazing losses. The different impact of zooplankton in this meso-oligotrophic region appears to be related to improved food quality and increased feeding effectiveness of the dominant zooplankton. The phytoplankton is dominated by smaller species of green algae, diatoms, and dinoflagellates. The zooplankton are dominated by larger crustaceans (*Daphnia galeata mendotae* and *Diaptomus*). The species and size composition of the phytoplankton and zooplankton in concert with the low, nutrient-limited (i.e. Auer et al. 1986) growth of the phytoplankton thus lead to a different dynamic interaction between the communities.

No temporal pattern in variation of grazing losses over growth gains for the phytoplankton at UB is evident in the data from the three summers. Sampling at closer intervals might reveal patterns. Others have reported grazing dominance associated with a brief clear-water period in inland lakes in spring (Sommer 1983; Lampert et al. 1986). Scavia and Fahnenstiel (1987) concluded that grazing plays a dominant role in algal dynamics in Lake Michigan during summer stratification when sinking losses were observed to be minimal.

At other times, *F* values at UB suggest conditions when grazing losses are less important to the phytoplankton. An indication of the variation in response of the phytoplankton to the full range of grazing rates is given in Fig. 4. UB data from all three summers on growth rate and chlorophyll biomass taken from specific depths of maximum volumetric productivity (p_{max} depths) are plotted against corresponding grazing rates from the same depths. The growth rate and chlorophyl biomass responses are similar and roughly take the form of a unimodal relationship in which maximum levels of growth and chlorophyll biomass are associated with intermediate grazing rates. This unimodal

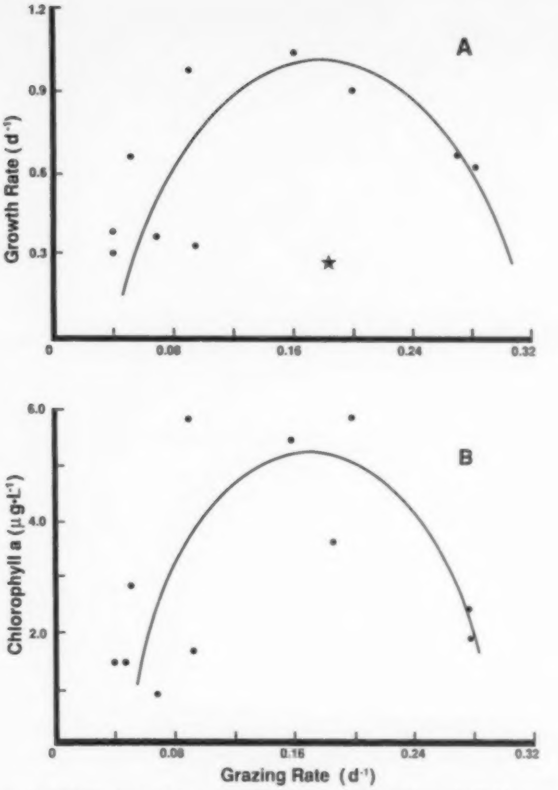

FIG. 4. (A) Phytoplankton growth rates and (B) chlorophyll biomass in relation to zooplankton community grazing rates at the UB sampling station on Green Bay. Data are from samplings in summers of 1986, 1987, and 1988 at the p_{max} depths. Lines are fitted by eye. The outlier data point (star) in Fig. 4A is excluded from the fit.

type response was predicted by Carpenter and Kitchell (1984, 1987). Empirical evidence for the unimodal response to increasing grazer biomass was first presented by Bergquist and Carpenter (1986) for particular algal species in 4-d mesocosm experiments.

Bergquist and Carpenter (1986) interpreted the response as resulting from contrasting grazer effects: stimulation of algal growth by nutrient regeneration at low grazer densities and reduction in algal biomass through grazing losses at high grazer densities. The responses in Fig. 4 of the two independent measurements, growth rate and chlorophyll biomass, to increasing grazing rates suggest that a similar interpretation can be made for the interactions occurring at the p_{max} depths at UB. Sterner (1989) noted that the positive grazer effect could also be attributed to thinning of the algal populations, thus preventing algae from monopolizing the nutrient resource pool. Either or both stimulatory effects of low grazing rates on the phytoplankton at UB may be occurring. These unimodal responses to community grazing measurements have not been reported previously for phytoplankton at the community level.

Trophic conditions at MB are intermediate although more similar to those at UB than at LB. Mean chlorophyll concentration at MB for the three summers is 6.00 $\mu g \cdot L^{-1}$ compared with 3.23 $\mu g \cdot L^{-1}$ for UB. A unimodal response of phytoplankton growth or chlorophyll biomass to increasing grazing rates was not observed at MB or at LB. Instead, weak inverse growth–grazing relationships were found at these more nutrient-enriched sites. Nutrient enrichment also altered the unimodal response observed by Bergquist and Carpenter (1986) to a weak inverse relationship between algal growth and grazer biomass.

Conclusion

In conclusion we note that the functional interaction of phytoplankton and zooplankton in Green Bay varies with trophic conditions. The numerical difference between phytoplankton growth rates and zooplankton grazing rates as an index (F) of the interaction increases with changing trophic conditions from northern to southern Green Bay; i.e. F is positively correlated ($r = 0.82$) with chlorophyll levels. Changes in F can be related to differences in the size distribution and species composition of both communities along the trophic gradient.

Eutrophic conditions produce large growth-grazing differences, averaging 0.56 for the water column and indicating dominance of algal growth in the interaction. Phytoplankton species are dominated by large-size cyanobacteria and zooplankton by small microcrustaceans.

Meso-oligotrophic conditions in the northern bay produce low and occasionally negative F values, averaging 0.08 for the water column and suggesting a more dominant role of the zooplankton. Larger cladocerans and calanoid copepods dominate the zooplankton. The phytoplankton are composed of small greens, diatoms, and dinoflagellates. Growth rates are about 28% those of the southern bay community reflecting the nutrient-limiting conditions of the northern bay area.

Both positive (growth stimulation) and negative (grazing losses) effects of grazers on the phytoplankton at UB are suggested by unimodal relationships of phytoplankton growth and chlorophyll biomass to increasing grazing rates. A unimodal phytoplankton response to grazing is not evident in the data for LB and MB. Higher ambient nutrient levels and algal growth rates possibly override any stimulatory effects of grazers at these stations.

Acknowledgments

This study was supported by the University of Wisconsin Sea Grant Institute (NA84AA-D-00065; R/GB-29). We thank C. Kraft, H. Harris, B. DeStasio, D. Jowett, G. Sprules, and R. Sterner for helpful comments. Technical assistance of D. Branstrator, K. Brown, E. Carey, T. Fairfield, L. Ganske, J. Gillis, M. Huber, K. Keating, D. Krueger, J. Krupka, and E. Sager is gratefully acknowledged.

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An Experimental Analysis of Macrophyte Growth Forms as Fish Foraging Habitat

Michele Dionne[1] and Carol L. Folt

Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA

Dionne, M., and C. L. Folt. 1991. An experimental analysis of macrophyte growth forms as fish foraging habitat. Can. J. Fish. Aquat. Sci. 48: 123–131.

In this laboratory study we measured the independent effects of macrophyte growth form, plant density, and prey abundance on the foraging rate of the pumpkinseed sunfish (*Lepomis gibbosus*). We demonstrate that macrophyte growth forms are not all similar in their effects on fish foraging. Prey capture rates of pumpkinseeds foraging among *Scirpus validus* (cylindrical stems) were 53 and 365% times greater than for *Potamogeton amplifolius* (leafy stems) for cladoceran (*Sida crystallina*) and larval damselfly (Coenagrionidae) prey, respectively. Plant growth form influenced prey capture rates more than charges in natural plant density. Plant density effects ranged from none on damselfly capture rates to a 29% decline in cladoceran capture rate over a twofold increase in plant density. Our results indicate that in plant-structured habitats, variation in plant growth form can be an important determinant of fish foraging and habitat associations.

Dans cette étude de laboratoire, nous avons mesuré indépendamment les effets de la forme de croissance macrophyte, de la densité végétale et de l'abondance des proies sur le taux de broutage du crapet-soleil (*Lepomis gibbosus*). Il est montré que toutes les formes de croissance macrophyte n'ont pas le même effet sur le broutage des poissons. Le taux de capture des proies du crapet-soleil broutant parmi *Scirpus validus* (tiges cylindriques) était plus élevé de 53 et 365 % que s'il broutait parmi *Potamogeton amplifolius* (tiges feuillues) pour la capture du cladocère *Sida crystallina* et de la larve de demoiselle (Coenagrionidae) respectivement. La forme de croissance des végétaux exerce un plus grand effet sur le taux de capture des proies que les variations naturelles de la densité végétale. Les effets de la densité végétale sur le taux de capture variaient de nul dans le cas de la demoiselle à une diminution de 29 % dans le cas des cladocères, la densité végétale étant doublée. Nos résultats indiquent que dans les habitats à structure végétale, la variation de la forme de croissance peut constituer un facteur important du broutage des poissons et des associations d'habitats.

Received December 6, 1989
Accepted July 24, 1990
(JA389)

Reçu le 6 décembre 1989
Accepté le 24 juillet 1990

The importance of physical plant structure to community interactions has been well recognized in terrestrial ecology (Goldberg and Werner 1983; Moermond 1979; Holmes and Robinson 1981; Robinson and Holmes 1984). In aquatic systems, as in terrestrial systems, plant species distribution patterns produce considerable structural variation across space within the littoral zone (Hutchinson 1975; Sand-Jensen and Søndergaard 1979; Chambers 1987; Chambers and Kalff 1987). Yet, despite the obvious importance of this variation, no study has examined how vegetation with different physical structure (or growth form) influences the ecology of fish inhabiting the littoral zone. Goldberg and Werner (1983) hypothesized that competitive interactions between plant species are determined primarily by plant growth form, and that different plant species with similar growth forms are ecologically "equivalent." We examine here the null hypothesis that macrophytes of two contrasting morphologies (cylindrical versus leafy stems) are equivalent with respect to their effects on fish foraging success. Specifically, we use simple laboratory experiments to (i) test whether fish foraging rates differ between two littoral zone plant species with contrasting growth forms and (ii) compare the effects of plant growth form with the effects of plant density on prey capture rates.

Thus far, macrophyte density is the only aspect of aquatic plant habitat structure that has been manipulated to measure its impact on fish foraging success. Foraging rates of piscivorous bass are inversely related to the density of man-made materials designed to simulate leafless cylindrical vegetation (e.g. wooden dowels, Glass 1971; or polypropylene rope, Savino and Stein 1982; Gotceitas and Colgan 1987). Similarly, sunfish foraging rates decline with an increase in the density of the structurally more complex species of macrophyte, *Ceratophyllum demersum* (Crowder and Cooper 1982). Diehl (1988) compared the effects of simultaneous changes in both density and form of two artificial plant types, finding that fish foraging rates on sediment-dwelling prey were reduced at high plant densities, even though the plant species used at high density had a simpler structure than the plant species used at low density. Together these studies indicate that the vegetation of the littoral zone can significantly affect fish foraging rates. Yet because none of these studies considered more than one type of vegetation for any one species of fish, the comparative effects of littoral vegetation types are not known.

Two major reasons exist to expect species-specific or growth form specific effects of macrophytes on fish foraging behavior. First, macrophytes can differ greatly in size, number, and orientation of leaves and stems; this variation may influence foraging success for both piscivores and invertebrate-feeding fish.

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[1] Present address: Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0406, USA.

TABLE 1. Comparison of field (Post Pond) and laboratory densities of experimental plant and prey species.

	Post Pond ($N=6$)			
	Mean	Range	SE	Laboratory
Plant species (plants·m^{-2})				
Potamogeton	34	2–48	9	16, 32
Scirpus	61	38–100	24	32, 64
Prey species (prey·cm plant length^{-1})				
Sida				
Potamogeton	1.730	0.660–2.780	0.880	0.25, 0.50, 1.00, 2.00[a]
Scirpus	0.008	0.000–0.160	0.007	
Damselflies				
Potamogeton	0.016	0.000–0.360	0.014	0.0420, 0.083, 0.167
Scirpus	0.022	0.011–0.058	0.018	

[a] Prey abundance (2 prey·cm plant length^{-1}) used only in *Potamogeton*.

TABLE 2. Actual numbers of plants and prey used as treatment levels for fish foraging experiments (see Table 1 for treatment levels expressed in same units as field samples). Differences in plant density levels between *Potamogeton* and *Scirpus* and in prey abundance levels between *Sida* and damselflies reflect differences in natural distribution and abundance at Post Pond. For each cell in the design, $N = 10$ fish.

	Prey abundance (prey per treatment) at plant density (plants per treatment):			
	Potamogeton[a]		*Scirpus*[b]	
	1	2	2	4
Prey species				
Sida	6			
	12	12	12	
	24	24	24	24
	48		48	48
Damselflies	1			
	2	2	2	
	4	4	4	4
			8	8

[a] This trial was carried out in October 1986.

[b] This trial was carried out in June 1987.

Second, prey abundance in the field also may vary with macrophyte species or growth form and in turn affect fish habitat associations and feeding rates.

In this study, two species of littoral plants are compared directly for their effects on aspects of sunfish foraging behavior. The aquatic plants *Scirpus validus* (thick, leafless stems) and *Potamogeton amplifolius* (thin stem, broad leaves) were chosen because both species are common littoral plants throughout North America (Ogden 1943; Fernald 1950; Gleason 1952) and because they possess distinctly different configurations of leaves and stems. The pumpkinseed sunfish (*Lepomis gibbosus*) is also common throughout eastern North America (Scott and Crossman 1973) where it feeds primarily in vegetated habitats (Mittelbach 1984). Two species of prey were used, the cladoceran *Sida crystallina* and larval zygoptera (Coenagrionidae). Both species are common littoral invertebrates with contrasting morphologies and behaviors; both contribute to the varied pumpkinseed diet (up to 10% by volume for *Sida* and up to 22% by volume for damselflies for fish beyond the young-of-the-year age class) (Keast and Welsh 1968; Keast 1978).

Methods

Experimental Design

We conducted two experiments to test for the effects of (i) plant growth form and possible interactions between plant growth form and prey abundance and (ii) plant density and possible interactions between plant density and prey abundance and to compare (iii) the results of plant density treatments at equal total prey with the results of plant density treatments at equal prey per plant and (iv) the results of plant growth form treatments with the results of plant density treatments.

The first experiment compared the effects of *Potamogeton amplifolius* and *Scirpus validus* on fish foraging for *Sida crystallina* (October 1986) and the second experiment compared the effects of the plants on fish foraging for damselfly (Zygoptera spp.) larvae (June 1987). We examined these interactions at natural field densities of plants and prey, based on a census of plant and prey densities in a local lake, Post Pond, NH (Tables 1 and 2). For this census, plant densities were estimated by counting individual plants in 0.25-m^2 quadrats placed at random points along transects within *Scirpus* ($N = 6$ quadrats) and *Potamogeton* ($N=6$ quadrats) stands at Post Pond in late summer. Natural abundances of invertebrate prey associated with the plants were estimated from samples collected on the same transects by drawing a plankton net (80-μm mesh, 30-cm diameter) from the pond bottom up under plants just as their stems were cut at the base.

Constraints of the experimental design did not always allow us to use prey abundances which fell within natural ranges. For example, to compare how *Sida* abundance influenced fish foraging between the two plant species, *Sida* abundances had to be the same for each plant species (Table 2). Because *Sida* was much more abundant on *Potamogeton* than on *Scirpus* (Table 1), higher prey abundances on *Scirpus* had to be used in the laboratory than are routinely found in the field.

Experimental Protocol

All fish were seined from a thickly vegetated backwater of the Connecticut River near the mouth of the brook that drains Post Pond. Fish were not collected from the pond itself to avoid damaging vegetation or disrupting fish censuses at our long-term study sites. The mean length (± SE) of the fish used in the experiment was 89 ± 5.2 mm standard length, which is close to the modal size for the Post Pond population (100 mm standard length). The same group of fish was kept at 20°C in an environmental chamber with a 12-h light–dark photoperiod for all experiments. Fish were housed individually in compartments 25 cm long × 25 cm wide × 30 cm high containing 15 L of water, with two compartments per 38-L aquarium. Aquarium size was chosen to maximize the number of aquaria, without apparently restricting aspects of fish foraging behavior. From field observations at Post Pond we know that pumpkinseeds tend to forage from plants for short periods (usually not more than 1 min) in small volumes (similar to the experimental compartments), before moving some distance away (often >1 m) and feeding again. Our experiments thus provide infor-

TABLE 3. Summary of main treatment effects on pumpkinseed capture and failed capture rates (by ANOVA). +, positive effect such that the variable increases with an increase in treatment level; −, negative effect such that the variable decreases with an increase in treatment level; ns, no significant effect. For example, under "Plant density effect" an increase in *Scirpus* density caused a decline (−) in the capture rate of *Sida*, but had no effect (ns) on damselflies. See "Data Analysis" in Methods, Table 4, and Fig. 1–5 for further explanation.

	Capture rate	Failed capture rate
Plant species effect		
Sida	*Scripus* > *Potamogeton*	ns
Damselflies	*Scirpus* > *Potamogeton*	ns
Plant density effect		
Potamogeton		
Sida	ns	ns
Damselflies	ns	ns
Scirpus		
Sida	—	—
Damselflies	ns	ns
Prey abundance effect		
Sida		
Potamogeton	+	+
Scirpus	+	+
Damselflies		
Potamogeton	−	ns
Scirpus	−	+

mation about short-term foraging behaviors, but not about fish movement between foraging areas.

Fish were maintained between experiments on a diet of 10 pellets per day of a commercial fish food (mean ± SD = 112 ± 13 mg for n = 20 rations of 10 pellets). Under this regime there was a mean increase in the initial wet weight of the fish of just 4.7% over 25 d.

All plants were collected from Post Pond and stored in aquaria under controlled light to maintain their natural epiphytic flora. They were placed in each tank on the first day of the trial, where they remained until they were changed on the first day of the next trial. Plants were evenly spaced in the tank and attached to the tank bottom with small transparent suction cups. Submerged plant length was equal to the depth of water in each tank (24 cm). Each *Potamogeton* plant was a single stem with six leaves (equivalent to the mean number of leaves per 24 cm of *Potamogeton* plant length at Post Pond). Each *Scirpus* plant was two leafless cylindrical stems that were joined at their bases, equivalent to the mean number of stems per *Scripus* plant at Post Pond. Just as the number of leaves per plant determine the growth form of *Potamogeton*, the number of stems per plant determine the growth form of *Scirpus*.

Prey were collected either at Post Pond, or from nearby backwaters of the Connecticut River. Intermediate size classes of each species were selected visually for use in the experiments. To test for significant differences in the size of the prey used between trials, *Sida* were subsampled (N = 90 to 144 per trial) and preserved in ethanol for later measurement, while damselflies were subsampled and measured live (N = 31 per trial). For *Sida*, mean body length ± SE was 1.81 ± 0.08 mm and for damselflies was 11.58 ± 1.26 mm. Mean body length did not differ among feeding bouts between trials (ANOVA, $F_{5,12} = 1.96$, $p > 0.10$ for *Sida*; $F_{4,10} = 0.07$, $p > 0.75$ for damselflies).

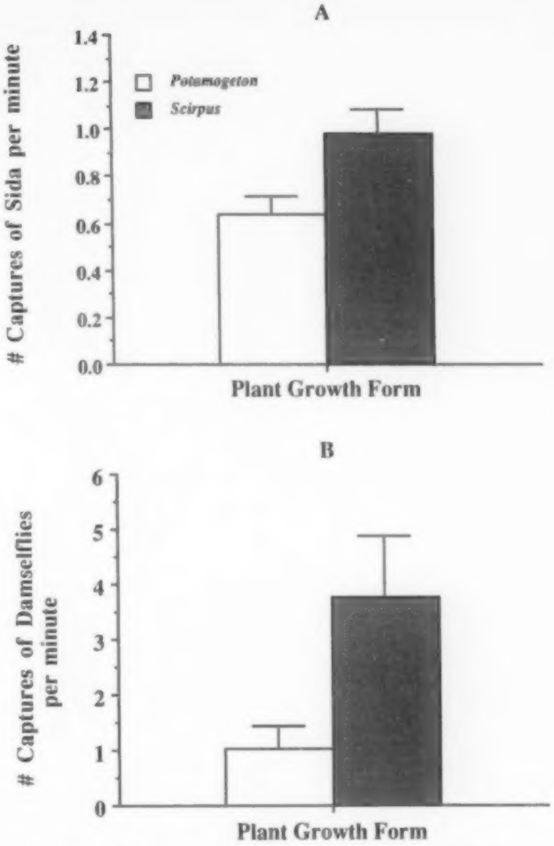

FIG. 1. Effect of plant growth form on pumpkinseed capture rates at plant density = 2·0.062 m^{-2} (area of tank bottom) across identical levels of total prey abundance (see Table 2). (A) p = 0.004 by ANOVA for plant growth form main effect when *Sida* were prey (F = 10.58, df = 1,18, n = 40). (B) p = 0.04 by ANOVA for plant species main effect when damselflies were prey (F = 4.78, df = 1,17, n = 39). Vertical bars indicate 1 SE.

Lighting in each tank was provided by a 15-W fluorescent light suspended 25 cm from the water's surface. Irradiance at the water's surface had a mean value ± SD of 14 ± 1.4 μeinsteins·m^{-2}·s^{-1} (n = 20). Unfortunately, figures are not available for irradiance in microeinsteins during the first several hours of sunlight (when pumpkinseeds feed most actively) at 1–2 m depth in vegetated habitats in natural waters. However, the experimental light level is within the lower range observed in shaded outdoor tanks, measured at 40 cm depth at 0800 (≈10 μeinsteins·m^{-2}·s^{-1} at 93% shade and ≈50 μeinsteins·m^{-2}·s^{-1} at 73% shade) (Barko and Smart 1981).

Fish were assigned to the experimental treatments to achieve a complete block design for tests within plant species (individual fish as blocks) and a split plot design for tests between plant species. Ten fish were randomly assigned to each plant species (growth form) treatment. Within plant growth form treatments, five combinations of plant density and prey abundance were randomly assigned to the 10 fish, with two replicate fish per treatment. Each foraging trial consisted of three replicate 5-min feeding bouts per fish for each assigned treatment. If the last prey was consumed before 5 min, the bout was terminated immediately. The interval between bouts for an individual fish

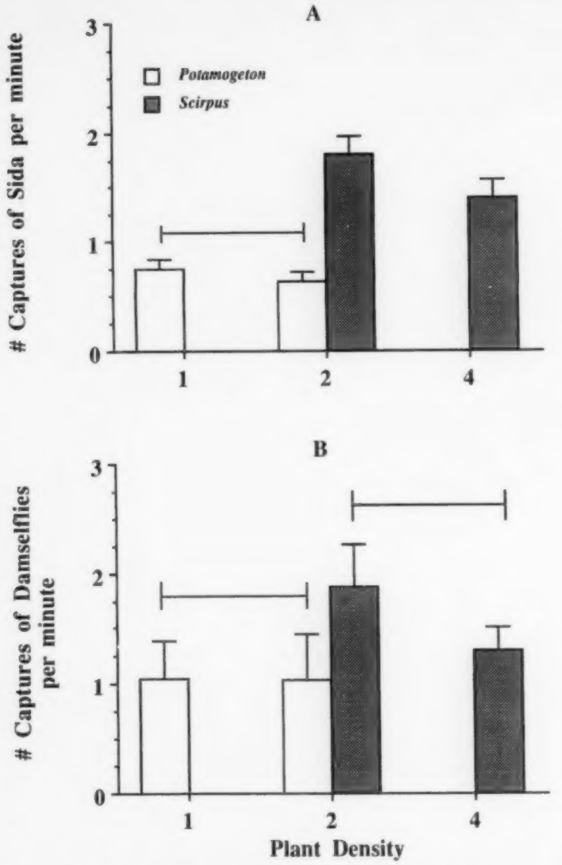

FIG. 2. Effects of plant density on capture rates across identical levels of total prey abundance (see Table 2). ANOVAs were performed separately for each plant species. Horizontal bars indicate main effects means which did not differ (within plant species). (A) $p = 0.144$ for *Potamogeton* density main effect ($F = 2.27$, df = 1,27, $n = 40$) and $p = 0.030$ for *Scirpus* density ($F = 5.28$, df = 1,25, $n = 38$) when *Sida* were prey. (B) $p = 0.965$ for *Potamogeton* density main effects ($F < 0.001$), df = 1,27, $n = 40$) and $p = 0.216$ for *Scirpus* density ($F = 1,61$, df = 1,25, $n = 38$) when damselflies were prey. Vertical bars represent 1 SE.

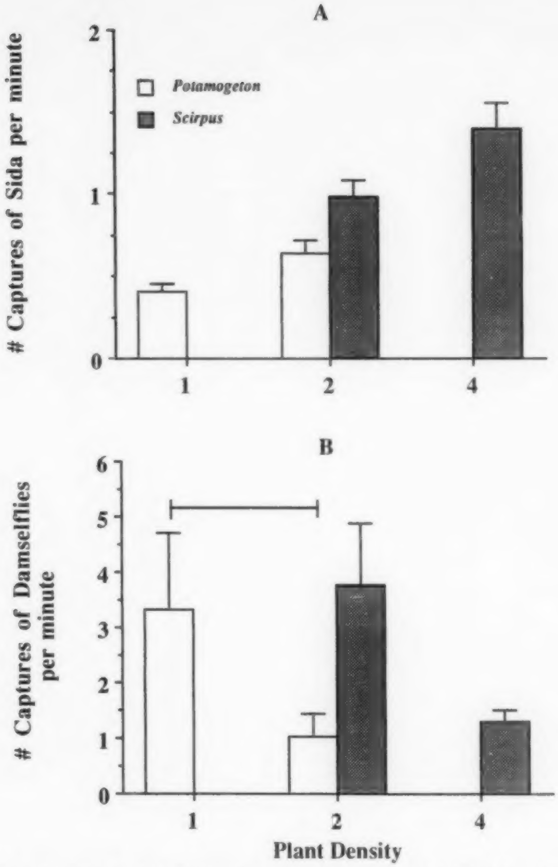

FIG. 3. Effects of plant density on capture rates for identical levels of prey abundance per plant (while total prey abundance necessarily increased with plant density). ANOVAs were performed separately for each plant species. Horizontal bars indicate main effects means which are not significantly different (within plant species). (A) $p = 0.002$ for *Potamogeton* density main effect ($F = 12.35$, df = 1,27, $n = 40$) and $p = 0.018$ for *Scirpus* density ($F = 6.39$, df = 1,26, $n = 39$) when *Sida* were prey. (B) $p = 0.12$ for *Potamogeton* density main effect ($F = 2.55$, df = 1,27, $n = 40$) and $p = 0.057$ for *Scirpus* density effect ($F = 3.98$, df = 1,26, $n = 39$) when damselflies were prey. Vertical bars represent 1 SE.

varied from 4 to 6 h. After completing feeding trials under the initial treatment conditions, experimental treatments were again randomly assigned until each fish received a treatment different from all those previously assigned to that fish. This process was repeated five times until each fish had received each treatment once and only once, resulting in replicate data from 10 fish for each of the five plant density – prey density treatment combinations.

A single trial without plants was conducted for each prey species (prey number = 8 for damselflies and 48 for *Sida*) to determine pumpkinseed feeding rates in the absence of plant structure, to account for any a priori differences in feeding rates between the fish assigned to the two plant treatment groups, and to detect any evidence of satiation at these high prey densities.

Fish were retained behind an opaque partition at one end of the tank before each feeding bout. For the week preceding each experiment, the fish were fed daily using the partitions, a diet consisting of 20 *Sida* and 2 damselflies plus the normal ration of commercial food. Plants were added to the tanks 3 d before the trial to accustom fish to their presence. On the day before the trial, fish were fed only commercial food. Each experiment began about 24 h after the last feeding. This procedure trained the fish to search for live food as soon as the partition was lifted, standardized their pretrial diets, and provided experience feeding on both prey species at the onset of each new experimental feeding trial.

Prey were added 15–20 min before each bout to allow them to settle on vegetation. Although our attempts to place individual *Sida* directly onto plants failed, the majority attached to plants by the end of settlement; others remained in the water column or attached to tank walls. There were no obvious differences in the proportion of prey attached to plant surfaces in the different plant treatments. Damselflies were introduced directly onto the plants and allowed to locate their own perch, where they remained until the start of the trial. After 15 min,

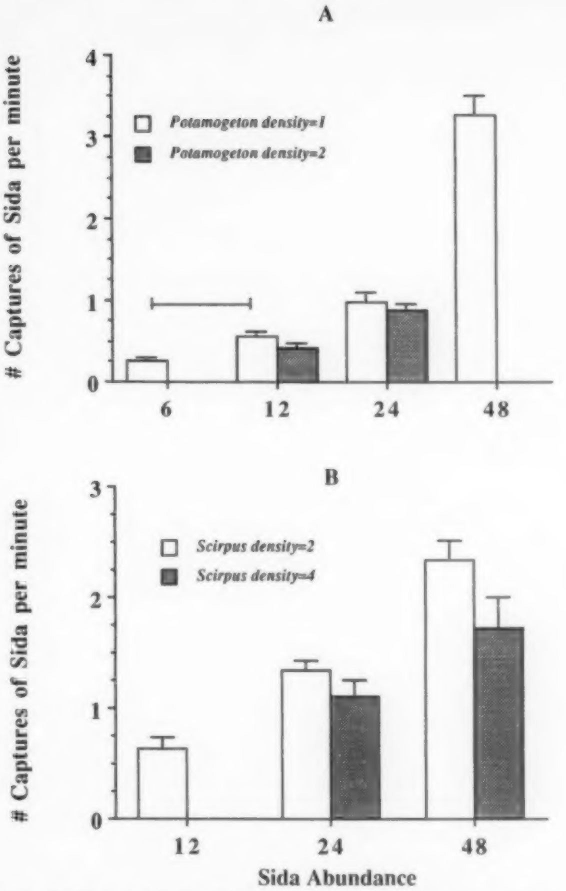

FIG. 4. Effects of *Sida* prey abundance on pumpkinseed capture rates. Horizontal bar indicates cell means which are not significantly different (within plant density), determined by Fisher's least significant difference test (SAS Institute, Inc. 1985). (A) $p = 0.0001$ for prey density main effects when plant was *Potamogeton* ($F = 130.08$, df $= 3,45$, $n = 60$). (B) $p = 0.0001$ for prey density main effect when plant was *Scirpus* ($F = 25.56$, df $= 2,34$, $n = 48$). Vertical bars represent 1 SE.

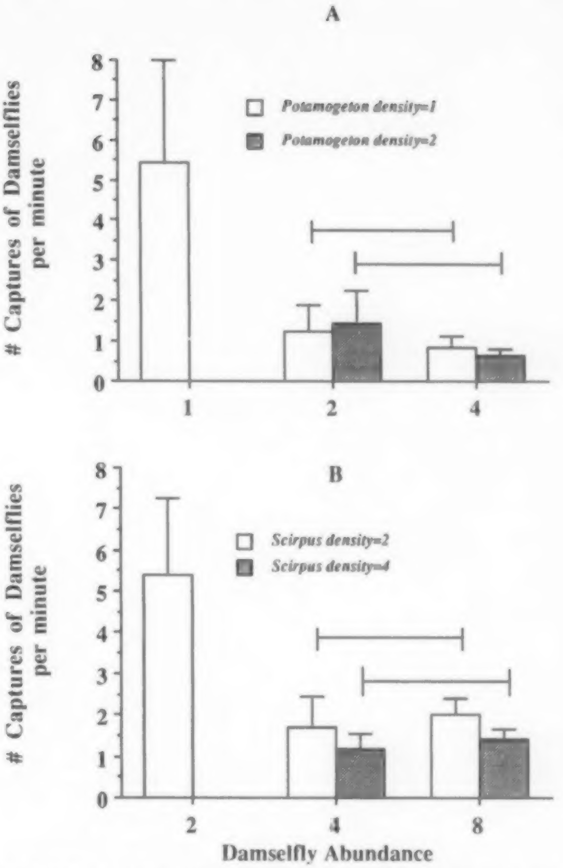

FIG. 5. Effects of damselfly abundance on pumpkinseed capture rates. ANOVAs were performed separately for each plant species. Horizontal bars indicate cell means which are not significantly different (within plant density), determined by Fisher's least significant difference test. (A) $p = 0.029$ for prey abundance main effect when plant was *Potamogeton* ($F = 3.93$, df $= 2,36$, $n = 50$). (B) $p = 0.013$ for prey abundance main effect when plant was *Scirpus* ($F = 4.97$, df $= 2,34$, $n = 48$). Vertical bars represent 1 SE.

damselflies were essentially motionless, and *Sida* activity in the water column (swimming from one attachment site to another) had declined to a constant, low level.

We began scoring the bout as soon as the partition was raised. Feeding behavior was recorded with an OS-3 Event Recorder™ (0.1-s precision). For each experimental fish we recorded prey captures and failed prey captures. Failed prey captures occurred when fish attacked but failed to ingest a prey, due either to inaccuracy in the attack or active movement by the prey.

Data Analysis

Data were analyzed to test for effects of plant growth form, plant density, and prey abundance for each prey species. With only one exception, no significant interactions occurred between plant species × prey abundance or plant density × prey abundance. Data were analyzed using fixed effects model analysis of variance (SAS Institute, Inc. 1985: Proc GLM, type III sums of squares). Analysis of residuals for capture rate and failed capture rate indicated general homogeneity of error variance and no serious departures from normality. In the several cases where data were transformed to improve homogeneity, interpretation of the results was not modified. Therefore we present results only from analyses of the original, untransformed data.

We also tested whether behavior changed (potentially due to habituation or learning) from the first to the third bouts of each trial. Bout effect was significant in only 2 of 30 analyses. These results indicate that the behavior in a given trial was independent of the bout in which it was observed. The bout term was subsequently removed from the models and all analyses were based on mean values across bouts for each dependent variable. This procedure ensured that the error degrees of freedom in the analyses were not inflated by including pseudoreplicates.

Results

The analyses of the plant growth form, plant density, and prey abundance treatments on two variables describing fish foraging behavior, capture rate, and failed capture rate are sum-

TABLE 4. Main effects means and results of ANOVAs for plant species, plant density, and prey abundance effects on failed capture rate. Effects of plant species were determined from identical levels of total prey abundance at a constant plant density, effects of plant density were determined from identical levels of total prey abundance, and effects of prey abundance were determined from identical levels of plant density (see Table 2). Means joined by a bar are not different ($p>0.05$ by Scheffe's test). Values in parentheses indicate 1 SE.

		Failed capture rate						
		Scirpus	*Potamogeton*		F	df	p	N
Plant growth form								
Sida		0.195 (0.042)	0.155 (0.038)		0.41	1,18	0.53	40
Damselflies		0.045 (0.023)	0.058 (0.024)		0.09	1,18	0.77	40
Plant density								
Potamogeton		1	2					
Sida		0.210 (0.038)	0.155 (0.038)		1.25	1,27	0.27	40
Damselflies		0.090 (0.042)	0.058 (0.025)		0.37	1,27	0.55	40
Scirpus		2	4					
Sida		0.389 (0.078)	0.212 (0.044)		5.11	1,25	0.03	38
Damselflies		0.093 (0.025)	0.100 (0.022)		0.05	1,25	0.83	38
Prey abundance								
Sida	6	12	24	48				
Potamogeton	0.036 (0.012)	0.128 (0.031)	0.236 (0.041)	0.444 (0.108)	9.70	3,45	0.001	60
Scirpus		0.174 (0.066)	0.198 (0.043)	0.415 (0.079)	7.18	2,34	0.003	48
Damselflies	1	2	4	8				
Potamogeton	0.001 (0.001)	0.052 (0.025)	0.097 (0.041)		1.44	2,36	0.25	50
Scirpus		0.024 (0.011)	0.053 (0.023)	0.137 (0.019)	8.07	2,34	0.001	48

marized in Table 3. Throughout the text, only comparisons of means with values of $p \leq 0.05$ are referred to as different.

Before vegetation was introduced to the tanks, foraging rates of the fish randomly assigned to the two different plant growth forms did not differ. Capture rates of *Sida* did not differ between the fish assigned to *Scirpus* (mean ± SE = $3.70 \pm 0.18 \cdot \mathrm{min}^{-1}$, $N = 10$) or *Potamogeton* (mean ± SE $3.61 \pm 0.30 \cdot \mathrm{min}^{-1}$, $N = 10$). Similarly, damselfly capture rates did not differ between fish assigned to *Scirpus* (mean ± SE $8.98 \pm 1.44 \cdot \mathrm{min}^{-1}$, $N = 10$) or to *Potamogeton* (mean ± SE $10.60 \pm 1.38 \cdot \mathrm{min}^{-1}$, $N = 10$).

Capture Rate

Capture rates were measured as the number of prey captured and ingested by pumpkinseeds per minute averaged over the entire bout. We tested for the effects of (i) plant growth form (simple *Scirpus* versus complex *Potamogeton*) and (ii) plant density on capture rate.

Capture rates differed between the two plant growth forms. At equal plant density and prey abundances, capture rates were higher in *Scirpus* than in *Potamogeton* for both prey species (Fig. 1; plant growth form effect, Table 3). Capture rates of *Sida* at equal prey abundances were reduced by a doubling of *Scirpus* density. However, *Scirpus* density did not influence capture rates of damselflies, nor did *Potamogeton* density influence capture rates of either prey (Fig. 2; plant density effect, Table 3).

The effect of plant density also was tested while holding the number of prey per plant constant (while total prey abundance necessarily increased with plant density). When analyzed in this way, capture rates of *Sida* increased with a twofold increase in plant density for both plant species. Capture of damselflies decreased in *Scirpus* ($p = 0.057$) and did not differ in *Potamogeton* (Fig. 3).

To analyze the effects of prey abundance (Fig. 4 and 5; prey abundance effect, Table 3), pumpkinseed capture rates were compared at equal plant densities. Capture rates of *Sida* increased with increased abundance of *Sida* in both *Scirpus* and *Potamogeton* (Fig. 4). Conversely, capture rates of damselflies decreased with an increase in damselfly abundances for both species of plant (Fig. 5).

Failed Capture Rate

Failed capture rate was measured as the number of prey attacks per minute in which prey were not ingested, averaged over the entire bout. It was used to assess the extent of interference of plant structure with capture attempts. Failed captures occurred at a very low rate, ranging from 0.036 to $0.444 \cdot \mathrm{min}^{-1}$ for *Sida* and from 0.001 to $0.137 \cdot \mathrm{min}^{-1}$ for damselflies across all experimental treatments (plant growth form, plant density, prey abundance). Failed capture rates did not differ between plant growth form for either prey species (Tables 3 and 4). As for plant density, fewer failed captures per unit time occurred for *Sida* as *Scirpus* density increased. Conversely, *Potamogeton* density had no effect. For damselflies, neither *Scirpus* nor *Potamogeton* density influenced failed capture rate. Lastly, in the prey abundance treatment, an increase in *Sida* increased failed capture rates in both *Scirpus* and *Potamogeton*. An increase in damselflies resulted in an increase in failed capture rates in *Scirpus* but not in *Potamogeton* (Tables 3 and 4).

Discussion

Macrophytes with contrasting growth forms provide different foraging habitats for pumpkinseed sunfish. The differences observed were great enough to suggest that the foraging rates of pumpkinseeds could vary significantly within and between lakes, depending on the composition of the littoral vegetation. Prey capture rates were greater in the stiff, straight-stemmed *Scirpus* than in the flexible, leafy *Potamogeton*. By comparison, a twofold increase in the density (similar to the natural density range) of either plant species did not influence capture rates in three of four comparisons. Thus we conclude that at natural densities, as found in our study site at Post Pond, plant density alone is less important than either prey abundance or a plant specie's growth form in determining prey capture rates.

In addition, differences exist in the degree to which plant growth form influences capture rates of *Sida* and damselflies. Capture rates in *Potamogeton* were reduced by 53 and 365% over capture rates in *Scirpus* for *Sida* and damselflies, respectively. The smaller magnitude of the plant growth form effect for *Sida* may be due to the small, translucent *Sida* appearing relatively cryptic on both plant surfaces, in comparison with the much larger damselflies appearing more conspicuous on the thin stems of *Scirpus* than on the broad leaves of *Potamogeton*.

Total prey abundance influenced capture rates in all cases, but the direction of the effect was a function of prey type: positive for *Sida* and negative for damselflies. The negative effect of damselfly prey abundance on pumpkinseed capture rates is probably due to a behavioral trait ("hiding") of the damselflies that we observed during the feeding trials (Dionne et al. 1990). We suggest that once hiding is triggered it reduces the capture rate by reducing encounter rate. Thus, at low prey density, prey were consumed before hiding was triggered whereas at high prey density the activity of pumpkinseeds capturing prey triggered hiding in the remaining prey.

Plant density affected capture rates more often when the number of prey per plant was held constant (and total prey abundance varied) than when total prey was constant (and prey per plant varied). The effect of plant density at constant prey per plant parallels the effects of prey abundance when plant density is held constant, i.e. capture rate increases with plant density for *Sida* and decreases for damselflies (in *Scirpus*). This change in the influence of plant density on capture rates when there is a constant number of prey per plant is actually due then to changes in total prey abundance.

Although several studies have examined the influence of aquatic vegetation on sunfish foraging under field conditions (Mittelbach 1981; Crowder and Cooper 1982; Werner et al. 1983b), no field study has measured the effect of plant density alone on foraging rates, due to the interdependence of plant density and prey density in nature (i.e. invertebrate prey abundance increases with plant abundance (biomass): Dvorak and Best 1982; Robertson 1984; Downing 1986; Cyr and Downing 1988). Crowder and Cooper (1982) proposed a model that included both of these factors as opposing forces, with increasing habitat complexity (equated with plant density) having negative effects on foraging rate through physical interference, and positive effects through the associated increase in prey density. The model predicts maximum fish foraging success at intermediate habitat complexity, where the negative effects of interference and the positive effects of prey abundance are both at intermediate levels. At low habitat complexity, there is little interference, but feeding rates are low due to the scarcity of prey. At high habitat complexity, prey are abundant, but feeding rates are low due to strong interference from the plants. Testing their model with a field experiment using bluegill sunfish and a single plant species, Crowder and Cooper (1982) found that fish at an intermediate plant density had highest growth rates, although the effects of prey and plant density were not measured separately.

As the Crowder and Cooper (1982) model suggests, we found that by increasing habitat complexity through plant growth form (without increasing prey abundance), fish foraging rates were reduced. However, we found no general negative effect of increasing habitat complexity through plant density (total prey abundance constant), using the natural range of densities from our field site. And, when plant density and prey density increased together (i.e. when the number of prey per plant was held constant), so did *Sida* capture rates. In the Crowder and Cooper (1982) model, this pattern would be expected at lower levels of habitat complexity, where foraging rate increases with complexity until the maximum rate is achieved, and beyond which further increases in complexity result in reduced foraging. Yet, given the minimal influence of plant density in our study, the pattern here may be a sole function of increased prey abundance. For damselflies, capture rate either declined or showed no change when plant density increased with constant prey per plant, suggesting that prey behavior may lead to fish foraging patterns that are not predicted by the model.

Our study separates the effects of plant density and prey abundance on fish foraging success and examines how the relationship between these factors varies for two morphologically distinct plant and prey species. The two levels of density used for each plant growth form ranged from slightly below the lowest measured field density to approximately two thirds of the highest field density. We feel that these densities represent natural density levels reasonably well. On one hand we do not wish to imply that the species and densities of plants that we have chosen are somehow representative of the variation in structure within and between all littoral habitats. On the other hand, since plant species with striking differences in growth form can have a considerable influence on fish foraging rates (this study), it should not be assumed that such differences among plants are unimportant.

The impact of growth form in nature, relative to plant density, may well depend upon the plant species composition of the habitat. Littoral habitats containing similar densities of macrophytes but very different growth forms may support very different levels of fish foraging. But when plant density varies by an order of magnitude, plant growth form may be less important than plant density. In habitats containing a more homogeneous mix of macrophytes, variation in fish foraging success may be better explained by the density and growth form of the constituent plant species than by total plant density alone. The *general* effects of plant architecture on fish foraging will not be understood until plant growth form effects are better studied.

The influence of plant growth form and plant density on pumpkinseed capture rates could have been due either to changes in the rates of successful captures (through effects on encounter rates) or failed captures (through physical interference). The failed capture analyses indicate that the observed prey capture rates were a direct outcome of changes in the rate of successful captures. The only significant effect of plant growth form or density on failed captures was a small decrease due to *Scirpus* density with *Sida* prey. However, the negative direction of this effect is the opposite expected from plant interference. Hence the physical structure of the plants influences the ability of pumpkinseeds to locate prey, but does not affect their ability to successfully attack prey once located.

Prey abundance also influenced failed capture rate. For *Sida*, both capture rate and failed capture rate increased with prey abundance, indicating that the rates of both successful and failed capture attempts were directly related to prey encounter rates. This pattern did not appear for damselflies because the only change in failed capture rate occurred between prey densities having no concomitant change in capture rate (prey = 4 and prey = 8 in *Scirpus*). In this case the increase in failed capture rate with prey density may be due to increased effectiveness in damselfly escape behavior, since at high prey density, more prey would be located in the vicinity of any given capture event. Fish capture activity may trigger changes in the behavioral state of neighboring prey that could in turn lead to the observed increase in failed captures.

Plant growth form influences fish foraging behavior in a number of ways. Prey visibility and location and fish foraging maneuvers may all be affected by vegetation type. Our study was designed only to relate the effects of plant growth form to plant density. Additional studies are required to determine the mechanisms responsible for the observed effects. Moreover, mechanisms determining the influence of plant growth form on fish foraging in the field may also involve differences in prey associations between growth forms.

For fish in the littoral zone, macrophytes provide not only foraging substrate for their invertebrate prey but also protective cover from predators (Glass 1971; Savino and Stein 1982; Werner et al. 1983a; Gotceitus and Colgan 1987; Werner and Hall 1988). As this study was restricted to the function of aquatic plants as feeding surfaces for a size class of pumpkinseeds that is invulnerable to the smallmouth bass (*Micropterus dolomieu*) and chain pickerel (*Esox niger*) piscivores at Post Pond, we did not attempt to assess the function of plant structure as refuge for pumpkinseeds. In systems where both predation and food can influence population growth, fish associations with specific plant habitats should be determined by the habitat's combined ability to provide both food resources and refuge from predation rather than a sole function of the habitat's quality as either a refuge or a foraging site.

The effects of plant species growth form on capture rate measured in the laboratory can lead to at least two alternate hypotheses concerning the outcome of these effects in nature. A plant of simple structure such as *Scirpus* that allows high capture rates in the laboratory may have the same effect in the field, providing superior foraging habitat relative to that provided by a plant species with a more complicated structure. Alternatively, the ease with which prey are captured in a structurally simple *Scirpus* habitat may lead to severely reduced prey standing stock and prey productivity. This may drive prey capture rates below that experienced in more complex habitats where the prey populations are not as readily reduced to low levels and total prey productivity remains higher. The results of our study demonstrate the lack of growth form equivalence among common littoral plant species, indicating that not only plant density but also plant growth form is an important factor determining fish foraging success in vegetated habitats. Further, they point to the importance of plant growth form in detemining the effects of natural habitat structure on fish growth and habitat associations. However, natural vegetated habitats vary not only in plant growth form but also in plant density and prey abundance (both plant-associated prey and benthic prey). The influence of these factors must be considered as well in any field study of the impact of plant growth form on fish foraging.

Species-specific effects of plant structure on fish foraging have not been previously considered, yet they have potentially broad implications for future studies of fish foraging in relation to aquatic habitat structure. Knowledge of the important contrasts between plant species in their suitability as fish foraging habitat would be of obvious use in efforts to restore or create new aquatic habitats for fish. We suggest that fish abundances or growth rates will often be influenced by the distribution patterns of favorable and unfavorable plant types. Furthermore, if the influence of plant growth form on foraging varies with fish species, then plant growth form is likely to be an important determinant of fish community structure in littoral habitats.

Acknowledgements

We thank the members of the ecology group at Dartmouth College for offering helpful comments and suggestions. P. Schulze, H. MacIsaac, J. Gilbert, R. Holmes, R. Stein, in addition to two external referees provided extremely helpful and thoughtful reviews of an earlier version of the manuscript. The study was supported by the Dartmouth College Cramer Fund and NSF BSR 8705480 to C.F.

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Relation between Mercury Concentrations in Walleye (*Stizostedion vitreum vitreum*) and Northern Pike (*Esox lucius*) in Ontario Lakes and Influence of Environmental Factors

C. D. Wren[1]

B.A.R. Environmental, R.R. 3, Guelph, Ont. N1H 6H9, Canada

W. A. Scheider

Water Resources Branch, Ontario Ministry of the Environment, 1 St. Clair Avenue West, Toronto, Ont. M4V 1K6, Canada

D. L. Wales

Fisheries Branch, Ontario Ministry of Natural Resources, Queen's Park, Toronto, Ont. M7A 1W3, Canada

and B. W. Muncaster[1] and I. M. Gray

B.A.R. Environmental, R.R. 3, Guelph, Ont. K1H 6H9, Canada

Wren, C. D., W. A. Scheider, D. L. Wales, B. W. Muncaster, and I. M. Gray. 1991. Relation between mercury concentrations in walleye (*Stizostedion vitreum vitreum*) and northern pike (*Esox lucius*) in Ontario lakes and influence of environmental factors. Can. J. Fish. Aquat. Sci. 48: 132–139.

We examined the relation between mercury levels in walleye (*Stizostedion vitreum vitreum*) and northern pike (*Esox lucius*) in Ontario lakes. Walleye and northern pike occurred together in 79 of the 346 study lakes. The length-standardized mercury concentrations in coexisting walleye and northern pike in the 79 lakes were highly correlated ($r^2 = 0.60$). The mean mercury concentrations in walleye and northern pike of standardized length from these lakes were 0.65 and 0.52 μg/g, respectively. Lacustrine characteristics associated with lake dystrophy (dissolved organic carbon and iron) were positively correlated with mercury levels in both species. Variables associated with acidity (pH and alkalinity) and hardness (calcium, conductivity, and magnesium) were negatively correlated with mercury concentrations in northern pike but not walleye.

Nous avons examiné la relation entre les concentrations de mercure présentes chez le doré (*Stizostedion vitreum vitreum*) et le brochet (*Esox lucius*) dans les lacs ontariens. Le doré et le brochet étaient tous deux présents dans 79 des 346 lacs étudiés. La corrélation entre les concentrations de mercure normalisées en fonction de la longueur chez le doré et le brochet cohabitant dans les 79 lacs était très élevée ($r^2 = 0{,}60$). Les concentrations moyennes de mercure chez le doré et le brochet de longueur normalisée de ces lacs étaient de 0,65 et 0,52 μg/g respectivement. Les caractéristiques lacustres liées à la dystrophie du lac (carbone organique et fer dissous) étaient corrélées de manière positive aux concentrations de mercure chez les deux espèces. Les variables liées à l'acidité (pH et alcalinité) et à la dureté (calcium, conductivité et magnésium) étaient corrélées de manière négative avec les concentrations de mercure dans le brochet mais non dans le doré.

Received December 8, 1989
Accepted July 24, 1990
(JA395)

Reçu le 8 décembre 1989
Accepté le 24 juillet 1990

Mercury concentrations in large fish from many lakes in Ontario without point sources of pollution often exceed the 0.5 μg/g consumption guideline (MOE/MNR 1988). It is clear that factors other than direct contamination from on-site sources influence mercury burdens in fish populations. For example, increased mercury levels in fish have been related to lake acidity (Brouzes et al. 1977; Bjorklund et al. 1984; Suns et al. 1987), organic content of the water (Verta 1984), flooding of drainage areas for hydroelectric reservoirs (Bodaly et al. 1984), and atmospheric deposition (Bjorklund et al. 1984; Johnson et al. 1986).

Several studies have shown strong correlations between tissue mercury concentrations in different fish species or other aquatic organisms within waterbodies or sampling locations. These studies include the following taxa: walleye (*Stizostedion vitreum vitreum*) and northern pike (*Esox lucius*) (Bishop and Neary 1976), lake trout (*Salvelinus namaycush*) and rainbow smelt (*Osmerus mordax*) (MacCrimmon et al. 1983), smallmouth bass (*Micropterus dolomieui*) and walleye (Wren 1988), yellow perch (*Perca flavescens*) and smallmouth bass (Suns et al. 1987), crayfish and smallmouth bass (Allard and Stokes 1989), filamentous algae and yellow perch (Stokes et al. 1983), crayfish (*Orconectes virilis*) and northern pike (Parks 1988), and walleye and yellow perch (Cope et al. 1990).

The high correlation observed between mercury concentrations in such diverse biota suggests that ambient environmental conditions within waterbodies influence the bioaccumulation of mercury. Knowledge of those parameters simultaneously influencing mercury levels in different aquatic biota will provide further understanding of the major environmental factors that govern the biogeochemical cycling of mercury.

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[1] Present address: Ecological Services for Planning Ltd., 361 Southgate Drive, Guelph, Ont. N1G 3M5, Canada.

Furthermore, if relationships in mercury concentrations between aquatic species can be accurately quantified, measurements of mercury in one species within a waterbody may permit estimation of mercury concentrations in other coexisting species. This will enable managers and agencies responsible for mercury monitoring programs to streamline their sampling regimes and make estimations from existing data.

Biota can accumulate mercury either directly from the water or food, and the relative importance of these two routes seems to be species and site specific (Phillips and Buhler 1978). Direct predator–prey relationships between pairs of species (e.g. crayfish and smallmouth bass or smelt and lake trout) in some of the studies emphasize the importance of the dietary mode of uptake. Waterbone concentrations of methylmercury directly influence mercury accumulation in aquatic biota (Rodgers and Beamish 1983; Parks 1988). However, Jackson (1988) reported that mercury levels in plankton and benthic invertebrates were not related to inorganic and methylmercury concentrations in water, but were strongly related to aqueous factors such as mercury-binding capacity.

The major objectives of this study were to examine the relation between mercury levels in walleye and northern pike in Ontario lakes and their correlation with physicochemical characteristics of the lakes.

Materials and Methods

Lake Selection

The Ontario Ministries of the Environment and Natural Resources have jointly developed a database incorporating mercury levels at standard fish length and lake physicochemical characteristics of Ontario lakes. Two hundred and fifty-five (255) lakes containing walleye and 170 lakes with northern pike were included. Seventy-nine (79) lakes contained both species. All the lakes contained naturally reproducing populations of northern pike and walleye.

The walleye and northern pike lakes were distributed widely across central and northern Ontario (Fig. 1). Both data sets ranged from approximately 76°00′W to 95°00′W and 44°30′N to 54°00′N. Both species occur throughout Ontario (Scott and Crossman 1973).

Length-Standardized Mercury Levels in Fish

A skinless, boneless fillet of dorsal muscle tissue was analyzed for mercury with cold vapour atomic absorption spectrophotometry at the laboratory of the Ontario Ministry of the Environment according to the procedures described in MOE (1984) and MOE/MNR (1988). The working detection limit by this technique is 0.01 μg/g, and the coefficient of variation on five or more replicate samples is less than ± 10%. All mercury concentrations are reported on a wet weight basis.

Individual lakes were included in our database if 10 or more fish were analyzed for mercury from that lake and a significant correlation ($p < 0.05$) existed between the logarithm of mercury concentration and total fish length (McMurtry et al. 1989). To eliminate any bias in fish mercury concentration between lakes due to differences in the size of fish sampled, the mercury concentrations were estimated for a hypothetical fish of a standardized length. Standardized total lengths of 41 and 54 cm were chosen for walleye and northern pike, respectively. These lengths represented sexually mature individuals capable of piscivory (Scott and Crossman 1973). The length range of fish sampled in most of our lakes contained fish of our standardized lengths. Fish were collected between 1978 and 1984 during the open-water season.

Lake Physical and Chemical Data

Twenty-two physical and chemical lake variables were included in the statistical analysis (Table 1). Physical parameters (lake area, elevation, maximum and mean depth, and morphoedaphic index) were obtained from the Ministry of Natural Resources Aquatic Habitat Inventory data set. Sample collection methods were outlined by Dodge et al. (1984). All water samples were taken from the centre of the lake as composite sample at a depth of at least 5 m, or 1 m above the sediment–water interface if the lake depth was less than 6 m.

Analytical methods for water chemistry variables were described by MOE (1984). Dissolved organic carbon and total kjeldhal nitrogen are abbreviated as DOC and TKN, respectively. For parameters with values below the detection limit we used one half of the detection limit in statistical analysis. The detection limits for chemical parameters were as follows: aluminum, 0.002 mg/L; chloride, 0.16 mg/L; iron, 0.02 mg/L; manganese, 0.002 mg/L; nitrogen, 0.02 mg/L; phosphorous 0.002 mg/L.

Water samples were taken between 1978 and 1988. However, all lakes with a complete listing of water quality data (Table 1) were sampled between February 12, 1988, and April 1, 1988. Fish were collected between 1978 and 1984, and there was often a time lag between the time of fish collection and water sampling. For example, for walleye lakes, the mean time span between water and fish sampling was 8 yr, with a range of 0–10 yr. Ninety-five percent of the water samples were taken after walleye were collected. For northern pike lakes, the mean time span between water and fish sampling was 6 yr, with a range of 0–10 yr. However, lake chemistry presumably did not change significantly within that time period. For example, Dillon et al. (1987) reported no significant changes between 1979 and 1985 in the chemistry of Harp Lake, which is more sensitive to acidic deposition than many of the lakes studied here (mean 1980–81 alkalinity = 3.7 mg/L, pH = 6.3).

Data Analysis

All statistical analyses were performed with Systat 4.0 (Wilkinson 1988). Lake chemical data were first examined for analytical or data entry errors by constructing ion balances. The balance was expressed as a ratio of the sum of the anions divided by the sum of the cations. The ideal balance is 1.0, while ratios between 0.85 and 1.15 were considered to be acceptable (APHA et al. 1976). Ion balances from four walleye lakes and two northern pike lakes were not within the ± 15% error range, and individual chemical outliers were removed from the data set.

Probability plots (values of a variable versus percentage points of a standard normal variable) and residual plots of each variable were examined for normality and homogeneity of variances, respectively. Plots were performed for all variables using raw values and natural logarithm, inverse, and square root transformations. Variables were transformed when the linearity of the probability plot was improved after the transformation. Transformed variables were indicated by prefixes (**ln** for the natural logarithm and **inv** for inverse) combined with the abbreviated variable name.

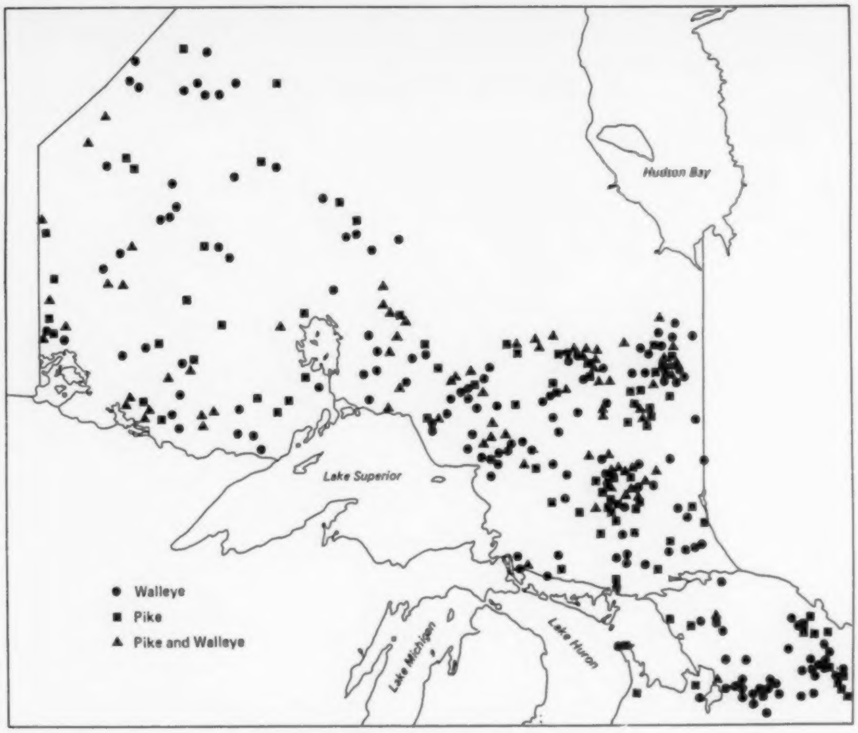

FIG. 1. Distribution of study lakes in Ontario.

The relation between length-standardized mercury concentrations in walleye and northern pike when these species occurred together in the same lake was examined by linear regression analysis. Parametric tests were used because assumptions of normality and homogeneity of variance were valid for the sample variables after transformation. Correlations between lake variables and predicted fish mercury levels were quantified with Pearson product-moment pairwise correlations.

Lake variables were entered against predicted fish mercury levels for a series of stepwise multiple regressions. The $\log_{10}$ of length-standardized fish mercury concentrations was used in all statistical analyses. Length-standardized mercury concentrations in coexisting walleye and northern pike ($n = 79$ lakes) were first compared with lake chemical and physical parameters. Finally, the relation between length-standardized mercury concentrations and lake parameters was examined separately for all walleye ($n = 255$) and all northern pike ($n = 170$) lakes.

For stepwise regression analysis, all lake variables were initially entered. Subsequently, a subset of lake parameters was selected to reduce problems due to correlations among independent variables. Several lake parameters including Secchi depth, iron, and DOC were highly correlated with one another. Individual variables for subsets in stepwise regression were chosen to represent chemical and physical characteristics of the study lakes (McMurtry et al. 1989). The traits represented included acidity (pH), dystrophy (DOC), geographical location (latitude), hardness (calcium), and lake size (surface area).

Results

Walleye and Northern Pike

Mean length-standardized mercury levels in walleye and northern pike from coexisting populations were positively correlated ($r^2 = 0.60$; Fig. 2). Within these lakes the mean length-standardized mercury concentration in 41-cm walleye (0.65 μg/g) was significantly greater ($p < 0.01$) than that in 54-cm northern pike (0.52 μg/g; Table 1). Lake pH and alkalinity were negatively correlated with logarithm of mercury concentration in northern pike, but not walleye (Table 2). Lake area was negatively correlated with mercury in walleye but not in northern pike. Water chemistry variables positively related to mercury concentrations in both species included DOC, aluminum, and iron (Table 2).

In stepwise regression with all variables, iron was consistently selected as the most important variable related to mercury burdens in walleye and northern pike. However, iron was highly correlated ($p \leq 0.05$) with certain other water quality variables including pH and DOC, so it is difficult to identify the causal factors affecting fish mercury levels.

Walleye

The overall mean length-standardized mercury concentration in walleye was 0.58 μg/g (range 0.09–3.24 μg/g; Table 3). Mean values and ranges of lake variables were similar for northern pike and walleye lakes with a few exceptions (Table 3). The mean area of lakes containing northern pike was 634 ha compared with 1413 ha for lakes with walleye. Several variables had very similar values for the two data sets. For example, mean pH (7.44 and 7.43) and calcium (15.3 and 15.1 mg/L) were similar in the two groups of lakes (Table 3).

The lake variables most highly correlated with mercury concentration in walleye were DOC, iron, Secchi depth, aluminum, TKN, nitrate, and lake area (Table 4). The positive coefficient between both DOC and iron and length-standardized fish

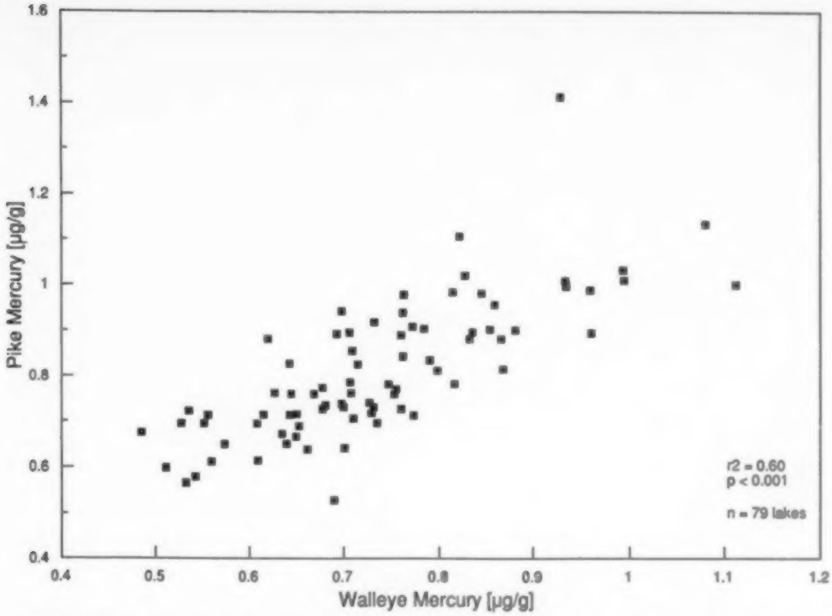

FIG. 2. Relation between length-standardized mercury concentrations in coexisting populations of walleye and northern pike.

TABLE 1. Sample size, mean, and range for numeric variables in walleye and pike overlap data sets.

Variable	*N*	Mean	Range	Abbreviation
Standard mercury (µg/g)				Hg
Walleye	79	0.65	0.23–2.22	
Pike	79	0.52	0.19–1.28	
Lake area (ha)	77	786.0	1.4–6579	LKAREA
Maximum depth (m)	78	20.5	1.5–76.2	Z_{max}
Mean depth (m)	78	5.4	0.6–20.6	Z_{mean}
Elevation (m)	78	315.0	194–458	ELEV
Secchi depth (m)	77	2.6	0.3–7.3	SECCHI
pH	62	7.47	6.48–8.19	pH
Alkalinity (mg/L)	63	50.6	4.2–126.1	ALK
Conductivity (µmho/cm)	59	110.2	30.0–246.0	COND
Chloride (mg/L)	48	0.69	0.10–4.60	Cl
Total kjeldhal nitrogen (mg/L)	48	0.34	0.18–0.74	TKN
Nitrate (mg/L)	48	0.08	0.01–0.27	N
Dissolved organic carbon (mg/L)	54	8.9	3.3–24.5	DOC
Calcium (mg/L)	55	16.3	3.0–37.9	Ca
Magnesium (mg/L)	56	3.61	0.76–8.52	Mg
Sodium (mg/L)	56	1.30	0.48–6.74	Na
Potassium (mg/L)	56	0.53	0.23–1.00	K
Phosphorus (mg/L)	49	0.004	0.001–0.023	P
Sulphate (mg/L)	55	4.62	1.26–9.00	SO_4
Aluminum (mg/L)	53	0.032	0.001–0.240	Al
Manganese (mg/L)	54	0.013	0.001–0.170	Mn
Iron (mg/L)	48	0.080	0.007–0.390	Fe
Morphoedaphic index	67	191	7–1151	MEI

mercury concentration indicated that mercury burdens in walleye were greater in lakes with high DOC and iron concentrations in the water. Iron, Secchi depth, maximum lake depth, and lake area were retained in stepwise regression with all variables (Table 5). Stepwise regressions on the smaller set of variables showed that DOC and calcium were retained (Table 5).

Northern Pike

The overall mean length-standardized mercury concentration in northern pike was 0.50 µg/g (range 0.07–1.28 µg/g; Table 3). Standardized mercury levels were negatively correlated with variables related to acidity (pH and alkalinity) and hardness (conductivity, calcium, and magnesium) (Table 4). These variables were also highly intercorrelated ($p \leq 0.05$). Examples of this intercorrelation included calcium and conductivity ($r^2 = 0.98$), magnesium and conductivity ($r^2 = 0.96$), and pH and alkalinity ($r^2 = 0.84$). These results indicate that higher mercury levels in northern pike were associated with low lake pH.

As with walleye lakes, DOC and iron were significantly positively correlated with length-standardized mercury concentra-

TABLE 2. Correlations of log-standardized mercury in pike and walleye with lake variables of the overlap data set. *Statistically significant at $\alpha = 0.05$; ** significant at $\alpha = 0.01$; ns, not significant.

	Pike		Walleye	
Lake variable	Pearson coefficient	*n*	Pearson coefficient	*n*
ln (AREA)	ns	77	−0.30**	77
pH	−0.27*	62	ns	62
ln (ALK)	−0.25*	63	ns	63
ln (DOC)	0.27*	54	0.27*	54
ln (Al)	0.41**	53	0.44**	53
ln (Fe)	0.48**	48	0.53**	48
SECCHI	−0.22*	77	−0.33**	77

tions in northern pike, although Secchi depth was not. Iron, manganese, and lake area were the only lake variables retained in a stepwise regression of standardized mercury levels in northern pike versus all lake parameters (Table 5). Calcium and DOC were two variables retained during stepwise regressions on the reduced set of variables similar to the walleye analysis (Table 5).

Discussion

Length-standardized mercury concentrations in walleye and northern pike were highly correlated. Other researchers have also reported correlations between mercury concentrations in different aquatic biota (Bishop and Neary 1976; MacCrimmon et al. 1983; Wren 1988; Suns et al. 1987; Allard and Stokes 1989; Parks 1988). However, this is the first report of correlations between mercury concentrations in fish species from a large number of geographically widespread lakes. The recognition of this relationship has implications for the design of fish contaminant monitoring programs. Knowledge of mercury levels in one species would enable estimation of mercury levels in other coexisting fish species.

The mean mercury content of walleye (0.65 μg/g) was higher than that of northern pike (0.52 μg/g) in lakes where both species occurred. Although we compared mercury levels in fish of standard length, the age of the fish was unknown. Thus, the observed difference in mercury concentration may be an artifact of our standard lengths chosen for these species. Mathers and Johansen (1985) reported that walleye accumulated more mercury and at a faster rate than northern pike within the same study lake. They suggested that walleye had a higher food consumption rate than northern pike and thus were exposed to more mercury in their diet. In contrast, Phillips et al. (1980) observed higher mercury concentrations in northern pike than in walleye of similar age in the Tongue River Reservoir, Montana.

Differences in diet between species could explain differences in mercury content between walleye and northern pike. Diet clearly influences mercury burdens in predatory fish (Phillips and Buhler 1978). MacCrimmon et al. (1983), for example, observed a sevenfold increase in the mercury content of stocked lake trout after a change in diet from benthic invertebrates to smelt. Mathers and Johansen (1985) reported that the diet of walleye in Lake Simcoe, Ontario, consisted mostly of smelt (68%) which contained higher mercury concentrations than the diet of northern pike which included only 8% smelt, with other forage fish making up the remainder.

The importance of lake variables in explaining predicted mercury levels in northern pike and walleye was species specific. Variables representative of hardness (calcium, conductivity, and magnesium) and lake acidity (pH and alkalinity) were negatively correlated with mercury levels in northern pike but not in walleye. Lakes with slightly lower pH were included in the northern pike data set (lower pH limit = 6.13) relative to the walleye data set (lower pH limit = 6.47). However, north-

TABLE 3. Sample size, mean, and range for variables in walleye and northern pike lakes.

	Walleye lakes			Northern pike lakes		
Variable	*n*	Mean	Range	*n*	Mean	Range
Hg (μg/g)	255	0.58	0.09–3.24	170	0.50	0.07–1.28
Lake area (ha)	244	1413	0.2–79 833	161	634.0	0.2–14 804
Maximum depth (m)	244	21.7	1.0–119.0	162	19.9	1.5–117
Mean depth (m)	242	5.8	0.4–37.6	162	5.5	0.6–29.6
Elevation (m)	244	304	140–465	162	306	92–480
Secchi depth (m)	235	2.9	0.2–9.3	156	2.7	0.3–7.3
pH	178	7.43	6.47–8.36	114	7.44	6.13–8.58
Alkalinity (mg/L)	177	44.2	1.91–143.3	113	48.9	1.9–154.8
Conductivity (μmho/cm)	174	105	30–318	107	112	25–312
Chloride (mg/L)	146	0.95	0.10–6.1	66	1.16	0.10–16.5
TKN (mg/L)	147	0.34	0.14–0.74	66	0.56	0.18–0.74
Nitrate (mg/L)	147	0.09	0.01–0.68	66	0.08	0.01–0.27
DOC (mg/L)	154	7.7	1.9–24.5	78	8.8	2.6–21.9
Calcium (mg/L)	158	15.1	2.9–42.1	84	15.3	2.0–38.5
Magnesium (mg/L)	159	3.38	0.74–9.78	85	3.50	0.76–8.52
Sodium (mg/L)	157	1.40	0.45–6.74	81	1.48	0.48–11.60
Potassium (mg/L)	158	0.64	0.20–2.12	82	0.56	0.23–1.36
Phosphorus (mg/L)	146	0.004	0.001–0.043	66	0.003	0.001–0.023
Sulphate (mg/L)	158	6.39	1.3–29	82	5.25	1.26–13.2
Aluminum (mg/L)	153	0.026	0.001–0.240	78	0.031	0.001–0.240
Manganese (mg/L)	154	0.013	0.001–0.170	76	0.010	0.001–0.050
Iron (mg/L)	147	0.069	0.004–0.390	66	0.083	0.007–0.390
Morphoedaphic index	202	193	7–1151	137	220	7–1647

TABLE 4. Significant correlations of log-standardized mercury with lake variables. * Statistically significant at $\alpha = 0.05$; ** significant at $\alpha = 0.01$.

	Walleye		Northern pike	
Lake variable	Pearson coefficient	n	Pearson coefficient	n
Latitude	0.13*	255	−0.27**	161
ln (AREA)	−0.25**	244		
ln (Cl)	−0.20*	146		
ln (TKN)	0.34**	147		
inv (N)	0.30**	147		
ln (DOC)	0.53**	154	0.32**	78
ln (SO_4)	−0.17*	158		
ln (Al)	0.40**	153		
ln (Fe)	0.47**	147		
SECCHI	−0.40**	235		
pH			−0.24**	114
ln (ALK)			0.22**	113
ln (COND)			−0.25**	107
ln (Ca)			−0.22*	84
ln (Mg)			−0.28*	85
ln (MEI)			−0.25**	137

ern pike mercury levels were also correlated with lake pH within the overlap data set where the pH range was the same for both species. The lake pH range observed in this study is undoubtably linked to the natural distribution of the species studied (Matuszek and Beggs 1988).

Studies in Wisconsin lakes reported a negative relationship between lake pH (pH range 5.0–8.1) and mercury concentration in walleye (Lathrop et al. 1989; Wiener et al. 1990). In contrast with this study, many reports of an inverse correlation between mercury in biota and lake pH involve a relatively small geographical sample area (Stokes et al. 1983; Wren and MacCrimmon 1983; Suns et al. 1987). Therefore, we examined the relation of mercury levels in walleye with lake pH from two subregions of Ontario. The subregions were Algonquin (78.5°W, 46°N; $n = 23$ lakes) and Gogama (81°W, 47.5°N; $n = 33$ lakes). Lake pH was negatively correlated with mercury in walleye ($r^2 = -0.43$) in the Algonquin region but not correlated ($p > 0.05$) with mercury in walleye in the Gogama region.

Negative correlations between lake pH and/or alkalinity and mercury content of aquatic biota have now been reported for filamentous algae (Stokes et al. 1983), yearling yellow perch (Suns et al. 1987; Sprenger et al. 1988; Grieb et al. 1988), pumpkinseed sunfish (Wren and MacCrimmon 1983), smallmouth bass (McMurtry et al. 1989; Suns et al. 1987), northern pike (Bjorklund et al. 1984; this study), and walleye (Lathrop et al. 1989; Wiener et al. 1990; this study). Rodgers and Beamish (1983) reported that the efficiency of methylmercury uptake by rainbow trout was much greater in softwater compared with hardwater.

It is not yet established if aqueous mercury (or methylmercury) concentrations are higher in low-pH lakes. Steffan et al. (1988) determined that production of methylmercury from anoxic sediments was not greater in acid lakes. However, reduced lake pH may increase bacterial methylation within the water column and at the sediment–water interface (Xun et al. 1987). Lower pH also decreases volatile loss of mercury from lake water and increases mercury binding to particulates in water (Winfrey and Rudd 1990). Therefore, elevated mercury concentrations in fish from low-pH lakes may be a direct function of higher mercury (or methylmercury) concentrations in water.

The lake dystrophy component (either DOC or iron) was significant for all stepwise regressions for both species. There is increasing empirical evidence to suggest that DOC is linked to mercury levels in fish. Fish mercury concentrations have been positively correlated with lake DOC levels in Ontario (Suns et al. 1987; Bowlby et al. 198; McMurtry et al. 1989). Studies in Sweden and Finland also noted a positive correlation between fish mercury levels and the humic content of water (Verta 1984, 1985; Lee and Hultburg 1990). Humic substances may act as both an energy source and source of mercury for the methylating bacteria (Bodaly et al. 1984; Verta 1985).

Mierle (1990) reported that total mercury concentrations were higher in brown water streams in southcentral Ontario than in clearwater streams. He suggested that humic materials can mobilize mercury from the watershed and act as a source of methylmercury to aquatic ecosystems. If water mercury concentrations are linked to DOC, this may explain the observed correlation between fish mercury and DOC observed in this and other studies. The relation between methylmercury concentrations in water and total mercury concentrations in biota is well established (Parks 1988).

There is increasing evidence that atmospheric deposition is a significant source of mercury to remote lakes (Rada et al. 1989; Meger 1986; Evans 1986; Johnson et al. 1986). Suns et al. (1987) reported a positive correlation between potential mercury loading (drainage area to lake volume ratio) and mer-

TABLE 5. Stepwise regressions with various lake variables entered versus predicted mercury levels in walleye and northern pike. Variables were significant at an enter and removal level of significance of 0.05. Partial R^2 values (R^2) are given for individual variables. Samples sizes are given in parentheses.

Walleye				Northern pike			
All variables (109)	r^2	Subset (150)[a]	r^2	All variables (50)	r^2	Subset (74)[a]	r^2
ln (Fe)	0.27	**ln** (DOC)	0.29	**ln** (Fe)	0.16	**ln** (DOC)	0.12
SECCHI	0.05	Ca	0.04	**ln** (Mn)	0.08	**ln** (Ca)	0.15
ln (Z_{max})	0.08			**ln** (AREA)	0.08		
ln (AREA)	0.05						
Model R^2	0.44		0.33		0.32		0.27

[a] Variables entered in subset were calcium, dissolved organic carbon, elevation, lake area, latitude, and pH.

cury levels in yellow perch. Johnson (1987) found a strong correlation between mercury loading rates and mercury concentration in a variety of fish species in Ontario lakes. Mierle (1990) suggested that at least 70% of the mercury entering a headwater lake in Ontario was derived from the atmosphere.

In this study, lake area was negatively correlated with mercury concentrations in walleye. However, DOC and lake area were negatively correlated ($p < 0.001$). Thus, the observed correlation between fish mercury levels and lake size may be due to a DOC effect rather than a lake size effect.

Differences in mercury burdens of fish may be explained by descriptors of water quality, especially dystrophy, acidity, and hardness. Thus, factors influencing these variables in Ontario lakes may also affect mercury availability and uptake in fish. Although significant relationships between mercury levels in fish and water quality have been reported in this study, it is still necessary to determine the causal relationships and mechanisms involved. Further research is also required to clarify the significance of atmospheric deposition and waterborne methylmercury concentrations to mercury availability in freshwater biota.

Acknowledgements

Financial support for this study was provided through the Ontario Ministry of the Environment Acid Precipitation In Ontario Study (AIPOS) program. M. McMurtry provided valuable input to the preparation of this manuscript. We are grateful for the review and comments on earlier drafts by B. Neary, G. Mierle, K. Somers, and A. Johnson.

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Nitrogen/Phosphorus Ratios and the Prediction of Chlorophyll in Phosphorus-Limited Lakes in Central Ontario

Lewis A. Molot

Faculty of Environmental Studies, York University, 4700 Keele Street, Toronto, Ont. M3J 1P3, Canada

and P. J. Dillon[1]

Ontario Ministry of the Environment, Dorset Research Centre, P.O. Box 39, Dorset, Ont. P0A 1E0, Canada

Molot, L. A., and P. J. Dillon. 1991. Nitrogen/phosphorus ratios and the prediction of chlorophyll in phosphorus-limited lakes in central Ontario. Can. J. Fish. Aquat. Sci. 48: 140–145.

The response of mean ice-free chlorophyll a in 15 stratified, P-limited oligotrophic and mesotrophic lakes in central Ontario to changes in mean epilimnetic total phosphorus (TP_{epi}) within a lake was highly variable between years during the period 1976–87. The linear regression coefficient of determination, R^2, using all annual means was only 0.36, and within-lake regressions revealed mostly random associations between chlorophyll a and TP_{epi}. Neverthless, by using the long-term average of annual means for each lake, a bivariate linear regression model was developed relating the long-term, average response of chlorophyll a to the long-term, average TP_{epi} concentration in these lakes ($R^2 = 0.78$). Annual variation could not be explained by changes in epilimnetic total nitrogen to total phosphorus ratio (TN/TP). The R^2 increased slightly from 0.78 to 0.82 with TN/TP as a second independent variable using long-term averages but remained at 0.78 with $1/TP_{epi}$ as a second variable. Reanalysis of published data excluding lakes which were not P limited showed that TN/TP is of little or no benefit as an independent variable. A minimum of six consecutive years of sampling was required to avoid anomalously poor fits (defined as $R^2 < 0.6$) for this set of lakes.

La production moyenne de chlorophylle a (absence de glace) dans 15 lacs stratifiés mésotrophiques et oligotrophiques du centre de l'Ontario et dans lesquels la croissance des végétaux était limitée par le P montrait, de 1976 à 1987, d'importantes variations annuelles en fonction de la concentration de phosphore total épilimnétique ($PT_{épi}$). Le coefficient de détermination de la régression linéaire, R^2, établi à partir de toutes les moyennes annuelles était de seulement, 0,36 et les régressions par lac indiquaient que les relations entre chlorophylle a et $PT_{épi}$ étaient le plus souvent aléatoires. Néanmoins, au moyen de la moyenne à long terme des moyennes annuelles pour chaque lac, un modèle de régression linéaire à deux variables reliant la réponse moyenne à long terme de la chlorophylle a à la concentration moyenne à long terme du $PT_{épi}$ ($R^2 = 0{,}78$) dans ces lacs a été élaboré. Les changements du rapport d'azote total à phosphore total (NT/PT) dans l'épilimnion ne permettent pas de rendre compte des variations annuelles. Le R^2 augmentait légèrement, soit de 0,78 à 0,82, avec NT/PT comme deuxième variable indépendante et les moyennes à long terme, mais est resté à 0,78 avec $1/PT_{épi}$ comme deuxième variable. Une nouvelle analyse de données publiées excluant les lacs non limités par le P a montré que l'utilisation du rapport NT/PT comme deuxième variable présente peu ou pas du tout d'intérêt. L'échantillonnage devait s'étaler sur au moins six années consécutives pour éviter d'obtenir des résultats anormalement pauvres (c'est-à-dire où $R^2 < 0{,}6$) pour cet ensemble de lacs.

Received February 29, 1990
Accepted July 23, 1990
(JA480)

Reçu le 29 février 1990
Accepté le 23 juillet 1990

A concerted effort has been made during the past two decades to predict the trophic status of lakes based on their response to total phosphorus (TP) loading and concentration (Vollenweider 1969; Dillon and Rigler 1975). Development of predictive tools has focussed upon steady-state models because parameterization of steady-state models is limited and because they are readily adaptable as management tools.

Two noteworthy models that have met with some success are those that predict chlorophyll *a* as a function of TP concentration in P-limited lakes (Dillon and Rigler 1974; Jones and Bachmann 1976; Nicholls and Dillon 1978; Canfield 1983; Prepas and Trew 1983; Riley and Prepas 1985; Dillon et al. 1988) and those that predict TP concentration as a function of annual TP load and flushing characteristics (Vollenweider 1969; Dillon and Rigler 1975; Dillon 1975; Kirchner and Dillon 1975; Larsen and Mercier 1976; Canfield and Bachmann 1981; Frisk et al. 1981; Chapra and Reckhow 1983; Reckhow and Chapra 1983). Together, these models permit dose–response estimates by linking land use activities in the catchment to changes in water quality.

To improve the goodness of fit (i.e. to better explain scatter in some data sets) between chlorophyll *a* and TP, linear regression analyses have included at times additional independent variables such as total nitrogen (TN) (Smith 1982; Canfield 1983), total nitrogen to total phosphorus ratio (TN/TP) (Canfield 1983; Dillon et al. 1988; Prairie et al. 1989), and mean depth (Pridmore et al. 1985). The argument that scatter can result from pooling data sets with different sampling and analytical methods (Nicholls and Dillon 1978) is typically minimized when large data sets are sought (Smith 1982; Canfield 1983; McCauley et al. 1989; Prairie et al. 1989).

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[1] Author to whom reprint requests should be addressed.

TN/TP has been included as a second independent variable because it is recognized that N is an important factor controlling standing crop in N-limited lakes and that the ratio is an indication of N or P limitation. Inclusion of the ratio may, therefore, permit construction of a regression equation which predicts chlorophyll *a* over a wide range of N and P limitation. Trophic response models must then include predictions of TN as well as TP — a major disadvantage. TN/TP has apparently improved goodness of fit even in data sets restricted to P-limited lakes (Dillon et al. 1988). This suggests that chlorophyll *a* is to some degree affected by TN/TP in P-limited lakes which is inconsistent with Liebig's Law of the Minimum (Odum 1971), laboratory evidence (Ahlgren 1988), and fertilization experiments (Schindler 1980).

We present here a statistical analysis of the relationship between chlorophyll *a* and TP in 15 P-limited lakes in central Ontario sampled from 1976 to 1987. The role of TN/TP as a factor controlling chlorophyll *a* is addressed as well as the effect of annual variation on regression goodness of fit.

Study Lakes

The study lakes are located in central Ontario in Haliburton County of the District of Muskoka. The catchments are primarily forested with some cottage development; details are provided in Dillon et al. (1987). They are underlain by Precambrian metamorphic silicate bedrock except for Glen Lake which is partially underlain by dolomitic marble. Mean depths range from 4.8 m in Gullfeather to 16.7 m in the main basin of Red Chalk Lake (Reid et al. 1987). All lakes thermally stratify but spring turnover is very incomplete in Glen Lake. The lakes are softwater (mean conductivity ranging from 28 to 39 μS except Glen and Basshaunt lakes which are 169 and 46 μS, respectively); all are oligotrophic to oligomesotrophic. Mean Secchi disc depth during summer stratification ranged from 2.8 m in Dickie Lake in 7.2 m in Solitaire Lake. Mean whole-lake alkalinity ranged from 10 to 130 μeq/L, except Glen Lake (1225 μeq/L).

Methods

Sampling procedures are described in Dillon et al. (1988) and all analytical methods are described in detail in Ontario Ministry of the Environment (1983). TN was calculated as total Kjeldahl nitrogen (TKN) plus nitrate (NO_3^-); nitrite (NO_2^-) levels were negligible. Total inorganic nitrogen (TIN) was calculated as NO_3^- plus ammonium (NH_4^+). Chlorophyll *a* was measured in volume-weighted samples representing twice the Secchi depth at least monthly and averaged within each lake for the summer stratification (Chl_{ss}) and ice-free (Chl_{if}) periods, the latter including both spring and fall overturn. Both Chl_{ss} and Chl_{if} were used in the analyses because standing crops during overturn could be important and because published regressions of data from these lakes have used Chl_{ss} (Dillon et al. 1988). TP was measured in volume-weighted epilimnetic, metalimnetic, and hypolimnetic samples, the depths of which were determined from temperature profiles. TP_{ss} refers to the whole-lake TP concentration and TP_{epi} refers to the epilimnetic TP concentration averaged for the summer stratification period.

Correlation and ordinary least squares regression analyses were performed with PC SAS using annual means as the basic "unit" parameter. Several regression analyses were performed: (1) chlorophyll *a* versus TP_{epi}, chlorophyll *a* versus TP_{epi} and $1/TP_{epi}$, and chlorophyll *a* versus TP_{epi} and epilimnetic TN/TP using long-term averages for each lake, (2) within-lake regressions of chlorophyll *a* versus TP_{epi} using annual data, and (3) within-year regressions of chlorophyll *a* versus TP_{epi} using annual data. The transformed variable $1/TP_{epi}$, lacks an a priori causal relationship with chlorophyll *a*. It was used as a reference for the variable TN/TP because of its similar construction yet arbitrary nature.

TABLE 1. Average (μg/L) of mean annual Chl_{ss}, Chl_{if}, TP_{epi}, and epilimnetic TN/TP (by weight) for the period 1976–87. Standard deviations are in parentheses; n = 12 yr for each lake except Red Chalk East (8) and Glen (11 for TP_{epi} and TN/TP and 9 for Chl_{if}).

Lake	Chl_{ss}	Chl_{if}	TP_{epi}	TN/TP
Blue Chalk	1.9 (0.2)	1.9 (0.2)	5.0 (0.6)	35.6 (5.9)
Chub	3.4 (1.1)	3.2 (1.1)	8.4 (1.2)	33.4 (4.9)
Crosson	2.8 (0.7)	2.6 (0.7)	9.1 (0.6)	30.7 (4.6)
Dickie	4.8 (1.0)	4.7 (1.3)	10.7 (2.1)	29.3 (7.7)
Harp	3.5 (0.7)	3.2 (0.5)	6.7 (0.7)	38.6 (6.2)
Red Chalk Main	2.3 (0.4)	2.1 (0.3)	4.4 (0.6)	48.1 (8.4)
Red Chalk East	2.8 (0.3)	2.6 (0.2)	4.8 (0.4)	48.1 (10.8)
Basshaunt	2.6 (1.0)	2.6 (1.0)	6.5 (0.9)	43.8 (8.9)
Bigwind	3.2 (1.0)	3.0 (0.8)	6.8 (0.6)	38.1 (6.8)
Buck	2.5 (0.5)	2.5 (0.6)	5.7 (0.6)	44.8 (7.8)
Glen	3.6 (1.7)	3.5 (1.4)	8.1 (1.1)	39.9 (7.0)
Gullfeather	4.6 (0.7)	4.4 (0.7)	11.8 (1.4)	29.0 (9.8)
Little Clear	2.9 (0.6)	2.8 (0.7)	6.3 (1.2)	40.4 (8.2)
Solitaire	1.9 (0.3)	1.8 (0.3)	5.0 (0.8)	43.4 (7.1)
Walker	2.9 (0.9)	2.7 (0.9)	5.8 (0.6)	48.3 (6.5)

When using the average of annual means, $1/TP_{epi}$ for each lake was calculated as the reciprocal of long-term average TP_{epi} and TN/TP was calculated as the long-term average of the ratios for each year with each ratio defined as (annual epilimnetic TN)/(annual epilimnetic TP).

Results

Annual Chl_{ss}, Chl_{if}, TP_{epi}, and epilimnetic TN/TP (by weight) were averaged for the period 1976–87 (Table 1). The lakes are oligotrophic to slightly mesotrophic with long-term mean annual TP_{epi} concentrations ranging from 4.4 to 11.8 μg P/L and Chl_{if} ranging from 1.8 to 4.7 μg/L. Long-term mean epilimnetic TN/TP was greater than 29 for all lakes. Mean ice-free TIN ranged from 73 to 175 μg/L (32 μg/L in Blue Chalk); hence, the lakes are considered to be P limited.

Chl_{if} and Chl_{ss} were, not surprisingly, similar and highly correlated, with pooled averages of all the lakes of 2.9 and 3.0 μg/L, respectively. The correlation coefficient was 1.0 using averages of annual means.

The correlation between TP_{epi} and Chl_{if} using long-term average, annual means was slightly higher (0.88) than the correlation between TP_{epi} and Chl_{ss} (0.86). Correlations between whole-lake TP_{ss} and Chl_{if} and between TP_{ss} and Chl_{ss} were substantially lower with $r = 0.64$ and 0.63, respectively, perhaps because the high TP concentrations in bottom waters do not contribute much to primary productivity. Consequently, only TP_{epi} was used in the following regression analyses.

Long-term Averages

The linear regression coefficient of determination, R^2 (the sum of squares due to regression/total sum of squares corrected

TABLE 2. R^2 for linear regression of chlorophyll *a* versus TP_{epi}, versus TP_{epi} and $1/TP_{epi}$, and versus TP_{epi} and TN/TP (by weight) using untransformed and logarithmically transformed data. Annual means for each of the 15 lakes were averaged for the two periods 1976–79 and 1976–87.

Independent variables	1976–79		1976–87	
	Chl_{ss}	Chl_{if}	Chl_{ss}	Chl_{if}
TP_{epi}	0.79	0.79	0.74	0.78
TP_{epi}, $1/TP_{epi}$	0.88	0.87	0.74	0.78
TP_{epi}, TN/TP	0.85	0.85	0.78	0.82
1976–87			log (Chl_{ss})	log (Chl_{if})
log (TP_{epi})			0.70	0.75
log (TP_{epi}), log ($1/TP_{epi}$)			0.71	0.76
log (TP_{epi}), log (TN/TP)			0.76	0.81

for the mean), for the 12-yr data set using the average of the annual mean values for Chl_{ss} versus TP_{epi}, versus TP_{epi} and $1/TP_{epi}$, and versus TP_{epi} and TN/TP was 0.74, 0.74, and 0.78, respectively (Table 2). Slightly higher R^2 values resulted from using mean Chl_{if} values rather than mean Chl_{ss} values for the 12-yr period (Table 2; Fig. 1A). Again, use of $1/TP_{epi}$ or TN/TP as additional independent variables was of little or no benefit.

Logarithmic transformations are commonly employed to linearize or normalize data. Logarithmically transforming the data resulted in lower R^2 (Table 2). The R^2 for log (Chl_{if}) versus log (TP_{epi}) was 0.75. Adding log ($1/TP_{epi}$) as an additional independent variable increased R^2 slightly to 0.76 and adding log (TN/TP) increased R^2 to 0.81. Similar results were obtained with log (Chl_{ss}).

Data from 1976 to 1979 were also analyzed for this set of lakes for comparison because Dillon et al. (1988) found, when using data from 1976 to 1979, that goodness of fit improved for Chl_{ss} when TN/TP was included as a second independent variable. Jerry Lake was excluded because it was not sampled after 1980. The R^2 for Chl_{ss} versus TP_{epi}, versus TP_{epi} and $1/TP_{epi}$, and versus TP_{epi} and TN/TP was 0.79, 0.88, and 0.85, respectively (Table 2). The R^2 using Chl_{if} was almost identical. The variable $1/TP_{epi}$ improved the regression fit better than TN/TP in the 1976–79 data set whereas $1/TP_{epi}$ had no effect in the 1976–87 data set. It is suspected, therefore, that the small increase in goodness of fit in the 1976–79 and 1976–87 data sets with TN/TP was due to "forcing" as a result of including additional variables.

Short-term Averages

The effects of annual variation on the chlorophyll *a* – TP relationship can be seen by using each lake-year as a separate observation (Fig. 1B). The R^2 for Chl_{if} versus TP_{epi} and log (Chl_{if}) versus log (TP_{epi}) was only 0.36 and 0.32, respectively ($N = 172$ lake-years). Scatter due to annual variation was not explained by the addition of secondary independent variables. The R^2 for Chl_{if} versus TP_{epi} and $1/TP_{epi}$, and versus TP_{epi} and TN/TP was 0.37 and 0.39, respectively.

The effects of annual variation are also seen in within-lake regressions of Chl_{if} versus TP_{epi} in which the mean R^2 was 0.13, ranging from 0.001 (Harp Lake) to 0.52 (Crosson Lake) (Fig. 2). The relationship in Crosson Lake, however, was inverse. Temporal variation in Chl_{if} in Harp and Crosson lakes was similar during some years, particularly 1979–83, whereas the temporal responses of TP_{epi} were not as similar (Fig. 3).

The effects of annual variation were also investigated by performing within-year(s) analyses. The R^2 for within-year (one year) regressions ranged from 0.11 (in 1986) to 0.79 (in 1978) (Table 3). A minimum of six consecutive years was required to avoid anomalously poor fits (defined as $R^2 < 0.6$) for this set of lakes because of one very low within-year R^2 in 1986. Even when the 1986 and 1987 years were excluded from the analyses, a minimum of four years consecutive sampling was necessary to ensure that $R^2 > 0.6$ and five years to ensure that $R^2 > 0.7$.

Discussion

There was little improvement in R^2 when TN/TP was included as a secondary independent variable in the regression analyses of long-term Chl_{if} versus TP_{epi} in the 1976–87 data set (4% increase; Table 2). In the 1976–79 data set, R^2 was higher with $1/TP_{epi}$ than with TN/TP, suggesting that mathematical/statistical "forcing" is as likely an explanation for the slight

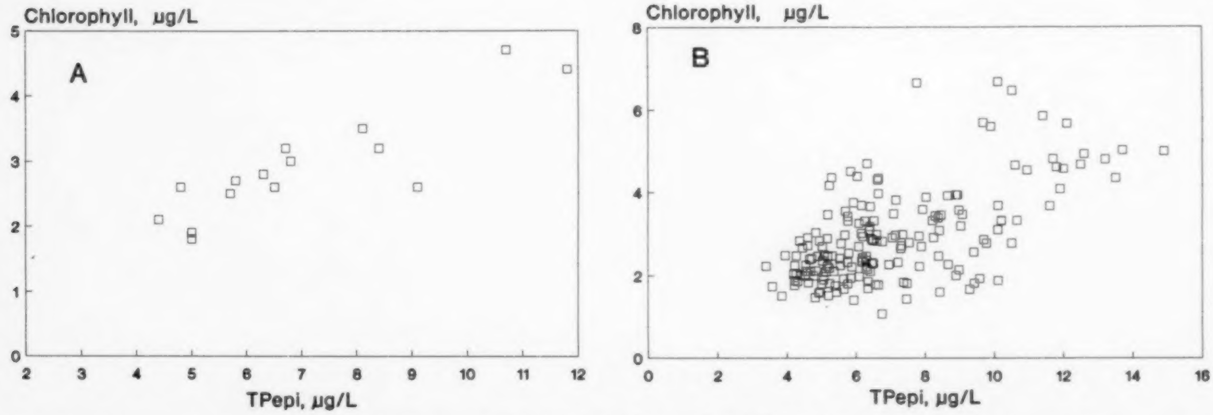

FIG. 1. Chl_{if} verus TP_{epi} for 15 P-limited lakes in central Ontario for the 12-yr period 1976–87. (A) Average of annual means. The regression equation is $Chl_{if} = 0.329\ TP_{epi} + 0.606$ ($n = 15$, $R^2 = 0.78$, slope SE = 0.048, and intercept SE = 0.353). (B) Annual means ($n = 172$ lake-years, $R^2 = 0.36$).

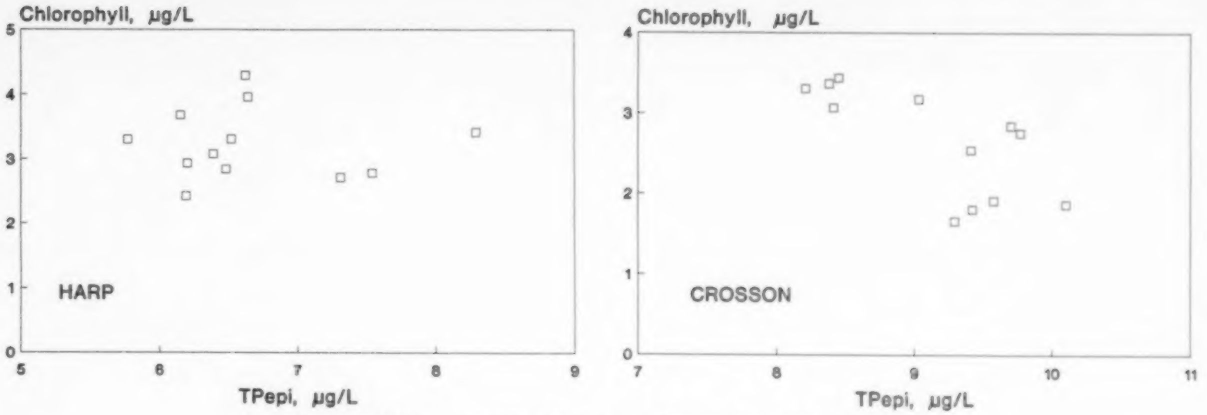

FIG. 2. Annual Chl_{if} versus TP_{epi} for Harp and Crosson lakes from 1976 to 1987.

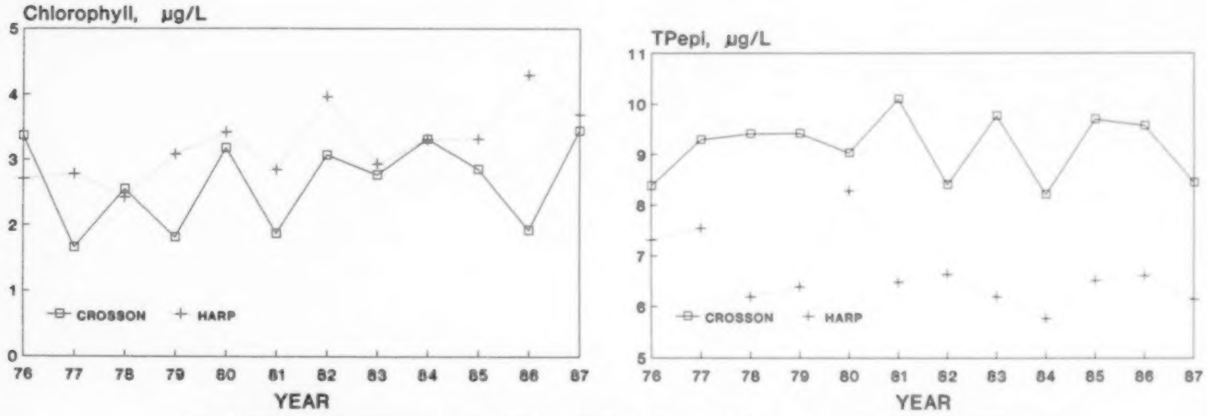

FIG. 3. Temporal responses of annual Chl_{if} and TP_{epi} in Harp and Crosson lakes from 1976 to 1987.

TABLE 3. R^2 range for linear regression of Chl_{if} versus TP_{epi} using running averages of annual means for the period 1976–87. n is the number of relationships derived from these data sets.

No. of consecutive years	R^2 range	n
1	0.11–0.79	12
2	0.29–0.80	11
3	0.40–0.80	10
4	0.50–0.79	9
5	0.54–0.82	8
6	0.63–0.77	7
7	0.66–0.80	6
12	0.78	1

increase in goodness of fit with TN/TP as is some causal explanation. A causal connection between TN/TP and chlorophyll *a* in these P-limited lakes is a weak one at best. It is doubtful whether TN/TP would have larger effects on lakes with higher TP concentrations provided the lakes are P limited.

Reanalysis of published data yields similar observations. Using average, summer values for stratified prairie lakes (Prepas and Trew 1983) with TN/TP ≥ 20, R^2 for chlorophyll *a* versus TP, versus TP and 1/TP, and versus TP and TN/TP was 0.73, 0.79, and 0.73, respectively. Reanalysis of data presented by Smith (1979) for 24 basin-years with TN/TP ratios ≥ 20 yielded R^2 values for chlorophyll *a* versus TP, versus TP and 1/TP, and versus TP and TN/TP of 0.71, 0.75, and 0.71, respectively. For eight stations with TN/TP ≤ 15 (7 were from one reservoir), R^2 was 0.56, 0.58, and 0.68, respectively (Smith 1979).

Pridmore et al. (1985) analyzed the log/log relationship between chlorophyll *a* and TP at 1 m depth in 21 New Zealand lakes and found that R^2 increased from 0.71 to 0.80 when the logarithm of mean depth was included. They subsequently recommended different regressions for lakes with mean depth less than or greater than 11 m. In reanalyzing their data, however, R^2 for untransformed chlorophyll *a* versus untransformed TP was 0.82, increasing slightly to 0.83 when mean depth was included as an independent variable. Hence, logarithmic transformations increased the variance which was subsequently attributed to a mean depth effect. Addition of TN/TP increased R^2 from 0.82 to 0.88 whereas 1/TP had no effect on R^2. When lakes with TN/TP < 20 were excluded, R^2 values for chlorophyll *a* versus TP, versus TP and 1/TP, and versus TP and TN/TP were identical at 0.94.

Pooling data for P-limited inland lakes (TN/TP ≥ 20) from Prepas and Trew (1983), Pridmore et al. (1985), and the central Ontario lakes (Table 1), R^2 for chlorophyll *a* versus TP, versus TP and 1/TP, and versus TP and TN/TP was 0.79, 0.83, and 0.79 ($n = 42$), respectively. Furthermore, logarithmically transforming the data lowered the goodness of fit with R^2 for log (chlorophyll *a*) versus log (TP) and versus log (TP) and log (TN/TP) of 0.54 and 0.58, respectively.

Clearly, analysis of central Ontario data and published data shows that TN/TP is of little or no use as a second independent variable in linear regression models of chlorophyll *a* versus TP in P-limited lakes. Fertilization studies provide corroborative experimental evidence by showing that chlorophyll increases are not affected by changes in loading TN/TP (Schindler 1980) or by N additions to P-limited enclosures (Prepas and Trimbee 1988).

There is laboratory evidence that dual limitation occurs in the transition zone between N and P limitation and therefore both N and P will affect phytoplankton abundance over a somewhat varying TN/TP range (the interactive model in growth kinetic studies) (Ahlgren 1988). However, beyond the transition zone, TN/TP is not expected to affect abundance.

The concept of attempting to predict chlorophyll as a function of TP in systems whose productivity is clearly not P limited is questionable. Although the shapes of published chlorophyll/TP curves are somewhat consistent, it does not follow that TP can or should be used as a major predictor variable when productivity is clearly not limited by TP (McCauley et al. 1989; Prairie et al. 1989; and others). When excess P occurs, either as luxury consumption or as high extracellular concentrations, quantitative relationships between chlorophyll and TP will weaken, resulting in increased scatter in chlorophyll/TP curves. The empirical approach to predicting chlorophyll has stressed TP under all limiting conditions; however, this approach is flawed and should be viewed with caution.

The response of Chl_{if} to changes in TP_{epi} between years within a lake was highly variable and appeared to be random (Fig. 1B and 2). Indeed, R^2 using all annual data was only 0.36, suggesting that annual differences in factors such as solar radiation, wind stress, water temperature, grazing rates (Pace 1984), etc., mitigate the response of phytoplankton communities to P. Annual variation could not be explained by changes in TN/TP.

Nevertheless, TP concentration clearly sets limits to growth about which other factors operate, i.e. fluctuations occur about an average, steady-state level. By using long-term means for each lake, a simple linear model was developed capable of predicting the long-term average response of chlorophyll *a* to the long-term average TP_{epi} concentration in oligotrophic and mesotrophic lakes with a relatively high R^2 (0.78). The wide range in standing crops produced at a given TP concentration reported in the literature (McCauley et al. 1989) is undoubtedly due in part to seasonal and annual variation — a feature of short-term data collection (Pace 1984). However, high correlations between fluctuating TP and chlorophyll within a summer season have been observed (Riley and Prepas 1984).

The response of chlorophyll *a* to TP_{epi} within the study lakes was due to a combination of regional and site-specific factors (Goldman et al. 1989). Strong regional influences affecting lakes were evident in the responses of Crosson and Harp lakes from 1979 to 1983 because the curves parallel each other whereas during other years, especially 1976 and 1986, lake-specific factors appeared to be dominant because the behaviour of the lakes appears to be independent (Fig. 3).

The extent to which lake-specific factors dominate may affect the extent of deviation from the regression equation in Fig. 1A. For example, Crosson Lake appears to deviate the most with a lower Chl_{if} than expected given its mean TP_{epi} of 9.1 μg/L. The R^2 for Chl_{if} versus TP_{epi} excluding Crosson was 0.89 compared with 0.78 using all lakes. The low standing crop in Crosson Lake may have been due to higher grazing pressure (Pace 1984). Alternatively, TP_{epi} may not be a good predictor of Chl_{if} in Crosson if significant quantities of biologically unavailable P are present. Perhaps goodness of fit could be improved using a chemical surrogate for biologically available P instead of TP (Butkus et al. 1988).

Reliable prediction of the long-term average response of chlorophyll *a* to epilimnetic TP in our study lakes was dependent upon an extensive sampling program involving frequent sampling during the ice-free season over at least six consecutive years. It was found that reliable prediction of average long-term TP export from 32 forested stream catchments also required about six consecutive years of sampling (unpublished data). Therefore, chlorophyll *a*/TP regression models based on surveys of P-limited lakes should be viewed as steady-state models with an inherent inability to predict annual variation. If short-term means are used (less than six years), goodness of fit may or may not be adequate. Long-term averages will ensure a higher goodness of fit. Although use of annual observations can reduce predictability over limited TP ranges, predictability will be less affected when large TP ranges are involved.

It should be noted that chlorophyll/TP models sometimes employ epilimnetic or trophogenic zone TP concentration whereas TP concentration/loading models (Vollenweider 1969; Dillon and Rigler 1974) employ whole-lake TP concentration. It should not be assumed that epilimnetic and whole-lake TP are similar. Average annual epilimnetic TP concentrations were less than whole-lake TP concentrations, ranging from 42% in Glen Lake to 95% in Dickie Lake. Therefore, whole-lake TP must be converted into epilimnetic or trophogenic zone TP in order to link models employing the two types of TP.

Ideally, a sound management policy for P-limited lakes, based on the relationship between chlorophyll *a* and TP, should be derived from data obtained from a multiyear, regional study using consistent methodology and excluding lakes which are not strictly P limited. Meeting these criteria will make it unnecessary to employ additional independent variables to improve goodness of fit, such as mean depth or TN/TP, and may make it unnecessary to transform data logarithmically. Meeting these criteria will, therefore, simplify modelling requirements.

Acknowledgements

We thank Ron Reid and Bob Girard for carrying out the field sampling programme and Barb Locke for assisting with the data analysis. This study was funded as part of the Ontario Ministry of the Environment and the Ontario Ministry of Municipal Affairs Lakeshore Capacity Study.

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Variance in Zooplankton Samples: Evaluation of a Predictive Model

Michael L. Pace, Stuart E. G. Findlay, and David Lints

Institute of Ecosystem Studies, Box AB, Millbrook, NY 12545, USA

Pace, M. L., S. E. G. Findlay, and D. Lints. 1991. Variance in zooplankton samples: evaluation of a predictive model. Can. J. Fish. Aquat. Sci. 48: 146–151.

We derived mean-variance relationships for zooplankton samples taken in the Hudson River and a series of Quebec lakes. Hudson River and Quebec lakes data were used to test a published model by comparing observed with predicted variance. In addition, we examined patterns of mean-variance relations (1) at higher levels of taxonomic organization, (2) between riverine and open water systems, and (3) between taxa common to the Hudson River and Quebec lakes data sets. There was an excellent correspondence between observed variance for the Hudson River and Quebec lakes data sets and the variance predicted using the model. Mean-variance relationships for higher taxa (cladocerans, copepods, rotifers) were similar to the general models. Zooplankton variance in the Hudson River was comparable with patterns observed in lentic systems contrary to our initial hypothesis that riverine zooplankton, because of rapid turbulent mixing, would have lower variance. Mean-variance relationships for taxa common to the Hudson River and Quebec lakes were comparable in three of six cases, but for the remaining three cases, regression intercepts were marginally different ($P = 0.03$–0.05) between systems. We conclude that zooplankton mean-variance relationships are highly consistent and predictable for a variety of aquatic systems and levels of taxonomic organization.

Nous avons établi des relations concernant la moyenne et la variance dans des échantillons de zooplancton recueillis dans la rivière Hudson et un ensemble de lacs du Québec. Ces données ont été utilisées pour évaluer un modèle déjà publié en comparant les variances observées et prédites par le modèle. De plus, nous avons examiné les relations existant 1) dans les taxons supérieurs, 2) entre le zooplancton de rivière et celui des lacs et 3) entre les taxons communs à la rivière Hudson et aux lacs du Québec. La correspondance entre la variance observée pour les données de la rivière et des lacs et la variance prédite par le modèle était très bonne. Les relations concernant la moyenne et la variance pour les taxons supérieurs (cladocères, copépodes, rotifères) étaient similaires à celles fournies par le modèle général. La variance du zooplancton dans la rivière Hudson était comparable à ce qui a été observé dans les systèmes lentiques contrairement à notre hypothèse de départ selon laquelle le zooplancton de rivière devait montrer une variance inférieure en raison de la forte turbulence. Les relations concernant la moyenne et la variance pour les taxons communs aux deux systèmes étaient comparables dans trois cas sur six; pour les trois autres cas, les coordonnées à l'origine n'étaient que légèrement différentes ($P = 0{,}03$–$0{,}05$). Nous concluons que ces relations sont très stables et prévisibles dans divers systèmes aquatiques et divers taxons de niveaux d'organisation différents.

Received March 20, 1990
Accepted July 23, 1990
(JA513)

Reçu le 20 mars 1990
Accepté le 23 juillet 1990

The variability of zooplankton at spatial, seasonal, and interannual scales has been an important focus of research because of interest in the (1) productivity, herbivory, and nutrient cycling by zooplankton in aquatic ecosystems (e.g. Makarewicz and Likens 1979), (2) biotic interactions of competition and predation in zooplankton communities (e.g. Vanni 1987), and (3) patterns and regulation of zooplankton population dynamics (e.g. Murdoch and McCauley 1985). Underlying the patterns of variation at scales of ecological interest is variation associated with sampling. Downing et al. (1987) presented a model which predicts the variance in replicate zooplankton samples as a function of the mean abundance and sample volume. This relationship provides a powerful tool because it allows an investigator to make a priori decisions about the amount of sampling necessary to achieve a given level of precision (Downing et al. 1987). As a consequence, tests of hypotheses about zooplankton are more feasible because sampling variation is predictable, allowing a better allocation of sampling effort.

The model of Downing et al. (1987) is based on data reported from published studies. Data were drawn from marine and freshwater systems and encompassed a variety of sampler types, sample volumes, and target taxa. The range of means used to establish the relationship covers 10 orders of magnitude. The heterogeneity of the studies and the large range of the data enhance the generality of the model. A remaining issue is how effectively the model predicts variance for specific independent data sets. Empirical relationships of this nature are often criticized for not being useful when applied to specific cases because of the logarithmic scales employed and wide confidence intervals (Peters 1986). Rarely, however, are established empirical relations subjected to tests in independent studies.

Here, we compare the Downing et al. (1987) model to variance-mean (hereafter s^2/m) relationships generated in two surveys using a common set of methods and a similar number of replicate samples. Zooplankton were sampled in a series of lakes in Quebec (Pace 1984, 1986) and in the Hudson River.

We extend the earlier analysis of Downing et al. (1987) in several ways. First, we ask whether s^2/m relationships are similar when species are aggregated into higher levels of taxonomic organization (e.g. rotifers, cladocerans, copepods). Surveys often consider zooplankton in this manner, and some hypotheses about zooplankton communities are tested at this scale (e.g. Pace 1986). Second, we examine whether variances in zooplankton samples drawn from the Hudson River are lower than variances observed in lakes and in the Downing et al. (1987) model which is based on open water marine and freshwater ecosystems. We hypothesize that the rapid and continuous mixing at both vertical and horizontal scales in the Hudson weakens behavioral responses of zooplankton to structure in their environment. Riverine zooplankton, therefore, would be expected to exhibit reduced patchiness, hence minimizing sampling variance. Finally, we present s^2/m relationships for individual taxa and compare relationships (lakes versus Hudson River) for common taxa to test whether taxon-specific s^2/m relationships are general across environments.

Methods

Study Sites

Twelve sites were sampled in lakes located in the Eastern Townships region of Quebec during 1982. These sites were chosen to represent a gradient of trophic and morphometric conditions (Pace 1984). Each site was sampled monthly during the ice-free season (May–September). Detailed descriptions of the sites and results were published in Pace (1984, 1986).

The Hudson River was sampled at six stations along the north–south axis of the river covering a distance of 158 km during 1987–89. Sampling stations and major characteristics of the Hudson River are described elsewhere (Cooper et al. 1988; Cole et al. 1990). An important feature of the Hudson in the region of our sampling is that the river is dominated by tidal flow (Cooper et al. 1988). There is essentially no vertical structure in temperature or chemical constituents with the exception of near-bottom increases in particles from resuspension (unpublished data).

Sampling

Triplicate zooplankton samples were taken using an open diaphragm bilge pump except for the May series of samples in the Quebec lakes which were vertical net hauls. For the pump samples, outflow was passed through a 70- to 80-μm-mesh net to sample macrozooplankton (primarily cladocerans and post-naupliar copepods) except for the 1987 Hudson River samples where we used a 150-μm net. Sampling volumes varied from 22 to 140 L in Quebec lakes depending on the lake, while in the Hudson River a constant sample volume of 105 L was used. In the Quebec lakes, separate triplicate samples were taken for microzooplankton (testate ciliates, rotifers, and copepod nauplii) using the pump and passing the outflow through a 35-μm sieve. Sample volumes varied from 13 to 45 L. In the Hudson, microzooplankton were sampled by collecting 2 L of water with a high-capacity peristaltic pump and filtering through a 35-μm net. Lake samples were integrated through the water column whereas river samples were from surface water only. Samples were preserved either in a sucrose–formalin solution or in the case of lake microzooplankton a saturated solution of $HgCl_2$.

Counting Procedures

To determine zooplankton abundance, either the whole sample was counted or at least 400 individuals were counted from subsamples of each replicate macrozooplankton and microzooplankton sample. Macrozooplankton were counted with a stereomicroscope (magnification 25–38 ×). Microzooplankton were settled in plankton chambers and counted with an inverted microscope (magnification 100–125 ×). Ciliates, rotifers, and cladocerans were identified at least to genus. Copepods were classified as cyclopoids, calanoids, or nauplii.

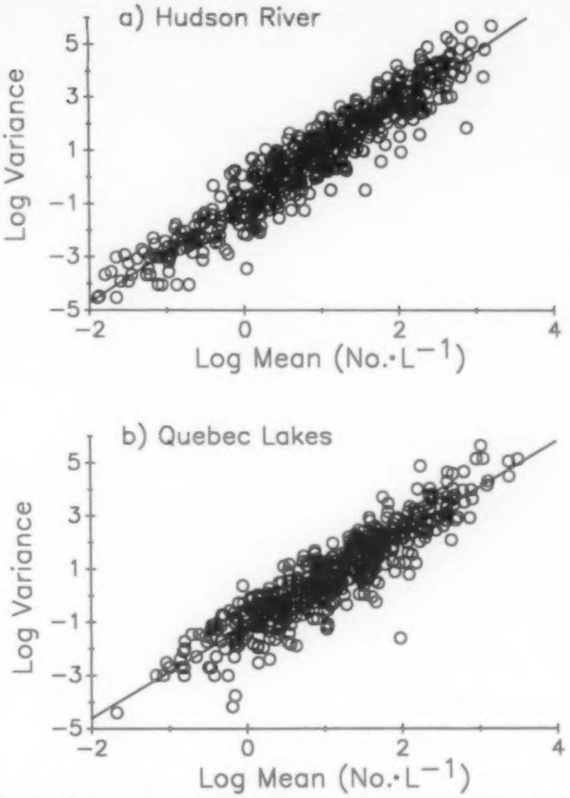

FIG. 1. Mean-variance relationships with least squares regression line for replicate zooplankton samples from the (a) Hudson River and (b) Quebec lakes.

TABLE 1. Regression coefficients and 95% confidence intervals (CI) for mean variance relationships fit to the model $\log s^2 = a + b \log m$, where s^2 = variance, m = mean number of organisms·L^{-1}, and a and b are fitted constants. Relationships for the taxonomic groups are derived from the QL data.

Model	a ± 95% CI	b ± 95% CI
Downing et al. (1987)	−0.528 ± 0.035	1.849 ± 0.011
HR	−0.931 ± 0.071	1.891 ± 0.052
QL	−1.130 ± 0.095	1.737 ± 0.066
Cladocerans	−1.305 ± 0.278	1.874 ± 0.232
Rotifers	−0.877 ± 0.827	1.626 ± 0.324
Copepods	−1.638 ± 0.466	1.796 ± 0.183

Results

Zooplankton in the Hudson River Compared with Quebec Lakes

Mean abundances of the three major groups of freshwater zooplankton (cladocerans, copepods, and rotifers) were substantially lower in the Hudson than would be predicted based on models developed for Quebec lakes (Pace 1986). For

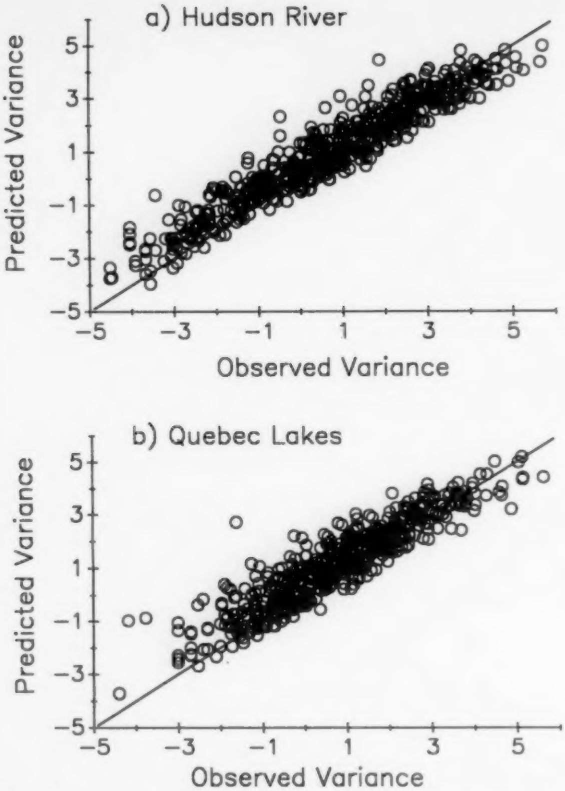

FIG. 2. Observed variance in zooplankton density estimates versus variance predicted by model employing mean density and sample volume (see text) from Downing et al. (1987) for the (a) Hudson River and (b) Quebec lakes. The 1:1 line is shown for comparison.

example, from May through September of 1988, mean densities of cladocerans, cyclopoid copepods, and rotifers observed at our most frequently sampled station were 4.8, 2.5, and 270 animals·L^{-1}. Given the average chlorophyll concentration at this station of 18 μg·L^{-1}, predicted densities based on Pace (1986) were 34, 38, and 1200 for cladocerans, cyclopoids, and rotifers, respectively.

Comparisons with the Model of Downing et al. (1987)

The s^2/m relationships for the Hudson River (HR) and Quebec lakes (QL) data (Fig. 1) are highly significant, with most of the variance in replicate samples explained by the mean (HR: $r^2 = 0.90$, $F = 5134$, $n = 567$; QL: $r^2 = 0.84$, $F = 2653$, $n = 517$). All models have slopes significantly >1 with negative intercepts (Table 1). In each case, $s^2 < m$ at low densities and $s^2 > m$ at high densities, indicating that the distribution of zooplankton at the replicate sample scale is not random ($s^2 = m$), but rather, depends on density.

The HR and QL models are similar to the relationship found by Downing et al. (1987) (Table 1). The fitted coefficients for the three relationships are highly specified because of the large number of samples and the good fit of the regressions. Consequently, 95% confidence intervals for the slopes and intercepts do not overlap with the exception of slopes for the HR and Downing et al. (1987) models (Table 1).

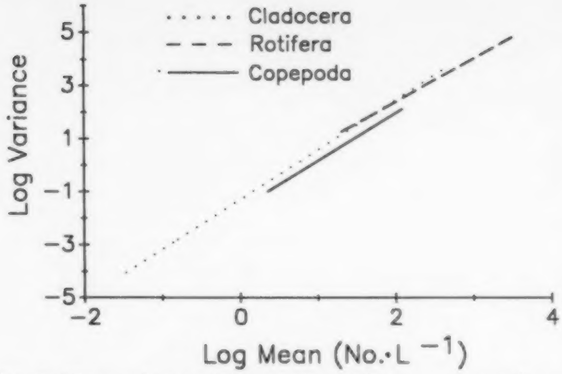

FIG. 3. Mean-variance relationships for cladocerans, copepods, and rotifers from the Quebec lakes.

TABLE 2. Mean residuals with 95% confidence intervals (CI) for cladocerans, copepods, and rotifers based on residuals for individual taxa in the QL regression.

Group	n	Mean residual	95% CI
Cladocerans	146	0.014	0.108
Copepods	114	−0.280	0.122
Rotifers	152	0.097	0.117

Differences among the models can be partly explained by the different sample volumes used. Downing et al. (1987) noted that variance declined with increasing sample volume and described a second model:

$$(1) \quad s^2 = 0.745m^{1.622}\,V^{-0.267}$$

where s^2 = variance, m = the mean number of organisms per litre, and V = volume of the sample (litres). There is an excellent agreement between observed variance and variance predicted from equation 1 in both HR and QL data sets (Fig. 2). Predictions are slightly biased with less variance observed than predicted at low densities and greater variance observed than predicted at high densities (Fig. 2). Nevertheless, the fit between observed and predicted values (HR: $r^2 = 0.91$, QL: $r^2 = 0.85$) and the tendency for a high density of points to fall on the 1:1 line (Fig. 2) support the ability of equation 1 to predict variance and aid in sampling design for specific situations.

Comparison of Models for Cladocerans, Rotifers, and Copepods

The relationships in Fig. 1 and Downing et al. (1987) are based on abundance estimates for species or genera of zooplankton with the exception of copepods which we identified only to major subdivisions (cyclopoids, calanoids, nauplii). To examine patterns for higher taxa, we developed s^2/m relationships for cladocerans, rotifers, and postnaupliar copepods based on the abundance estimates for these groups from the QL data (Fig. 3). In the freshwater Hudson River, one species of copepod (*Diacyclops bicuspidatus thomasi*) and one cladoceran (*Bosmina longirostris*) dominate, so relationships for higher order taxa drawn from HR data would be largely based on a single species and inappropriate for our purposes.

Regressions for cladocerans, copepods, and rotifers are quite similar to the overall QL model and to the Downing et al. (1987) relationship (Fig. 3; Table 1). Copepods occurred over a nar-

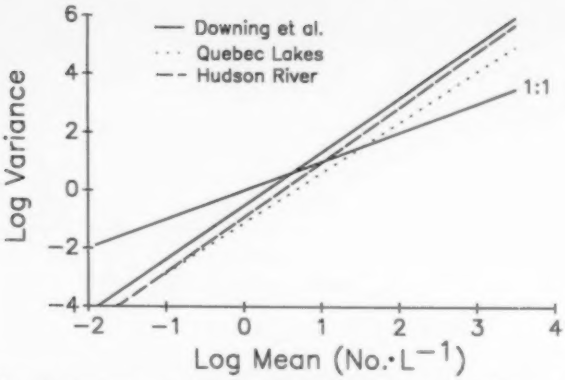

FIG. 4. Comparison of the regresion models from Downing et al. (1987), the Hudson River, and the Quebec lakes. A 1:1 line illustrates the s^2/m relationship if variability followed a Poisson distribution.

rower range of densities and tended to have lower variance across the range of densities than observed for cladocerans and rotifers (Fig. 3).

To further compare s^2/m patterns for cladocerans, copepods, and rotifers, we calculated residuals (the difference between observed and predicted s^2) for each lower taxon in the QL model of Fig. 1b, and mean residuals were determined for each group of taxa. The mean residuals for cladocerans and rotifers were near zero whereas the mean for copepods was distinctly lower (Table 2). Heterogeneity among group residuals, tested with a one-way ANOVA, was significant ($F = 10.3$, $P < 0.0001$, $n = 3$). Individual copepod taxa (in this case, calanoids and cyclopoids) have a significantly lower variance per unit mean abundance in the QL data than rotifers and cladocerans (Duncan's multiple range test, $P < 0.05$).

Variability of Riverine Zooplankton

To test our hypothesis that zooplankton in the Hudson River are less patchy due to vigorous mixing, we compared the HR model with the QL and Downing et al. (1987) models. A lower slope for the HR s^2/m relationship relative to the QL or the Downing et al. (1987) model would indicate that zooplankton are less variable as density increases in the Hudson. A lower intercept would indicate that zooplankton are less variable per unit mean abundance. Neither of these patterns were evident when comparing the models. The slope for the HR data is similar to the Downing et al. (1987) slope and higher than the QL slope (Table 1). The HR intercept, although lower than the Downing et al. (1987) model, is higher than the QL model (Table 1). When the three regressions are plotted together (Fig. 4), the HR line is intermediate between the two lines derived from nonriverine systems. There is no evidence based on this comparison that the patterns of s^2/m relationships are related to the degree of mixing in a particular environment.

Taxon-Specific s^2/m Relationships

Relationships for individual taxa were always highly significant ($P < 0.0001$) with slopes (b) falling in the range of 1.2–1.9 (Table 3). All slopes with the exception of *Holopedium* and nauplii (QL data) were significantly greater than 1 ($P < 0.05$), indicating a positive nonlinear increase in variance with density for individual taxa (Table 3). For the HR taxa, r^2 ranges from 0.69 to 0.93. Scatter in the taxon-specific relationship for the QL data was greater, with half of the r^2 values being <0.6 (Table 3).

TABLE 3. Summary of taxon-specific relationships for HR and QL data sets. Model and coefficients are as in Table 1. For all regressions, $P < 0.0001$.

Taxon	a	b	r^2	n
		HR		
Bosmina longirostris	−0.964	1.852	0.93	127
Cyclopoids	−1.221	1.666	0.74	138
Keratella spp.	−1.041	1.876	0.69	69
Nauplii	−0.292	1.519	0.73	68
Polyarthra spp.	−0.204	1.608	0.80	59
Tintinnopsis spp.	−0.062	1.533	0.77	60
Trichocerca spp.	0.026	1.390	0.77	46
		QL		
Bosmina spp.	−1.209	1.843	0.77	57
Calanoids	−1.089	1.407	0.54	55
Cyclopoids	−1.361	1.541	0.69	59
Daphnia spp.	−0.912	1.613	0.83	54
Holopedium gibberum	−1.355	1.418	0.46	35
Kellicotia longispina	−0.627	1.544	0.54	36
Keratella spp.	−0.819	1.622	0.81	58
Nauplii	−0.284	1.217	0.39	59
Polyarthra spp.	−1.138	1.719	0.58	58
Tintinnopsis lacustris	−0.763	1.692	0.80	47

In comparing the taxon-specific relationships, we examined whether the regression coefficients were influenced by either the number of s^2/m pairs (n) used in the regression or the range of abundances observed. If either intercepts (a) or slopes (b) are a function of the number of samples or range of abundance observed, comparisons between s^2/m relationships are problematic (Downing 1986). There were no significant correlations between n and range and the regression coefficients, a and b, for the 17 taxon-specific relationships in Table 3. The independence of the regression coefficients from n, range, and the similar number replicate samples (three) taken in each case allow us to compare regressions between the two studies and determine if taxon-specific models tend to be similar across environments.

We compared taxon-specific models using ANOVA. Data for a taxon common to both environments (e.g. *Bosmina*) were fitted to the model

$$(2)\quad \log s^2 = a + b_1 \log m + b_2\,\text{study} + b_3 \log m\cdot\text{study}$$

where study takes a value of 0 or 1 for HR and QL studies, respectively. Significant regression coefficients for b_2 and b_3 imply significant differences between intercepts and slopes, respectively, of the two regressions (Gujarati 1978). Intercepts (b_2) were marginally different between the studies for *Bosmina* ($P = 0.04$), *Polyarthra* ($P = 0.03$), and *Tintinnopsis* ($P = 0.05$), but there was no difference for cyclopoids, nauplii, and *Keratella* ($P > 0.05$). Slopes were not significantly different for any of the six taxa compared. We conclude that variation in taxon-specific models is modest but potentially significant across environments.

Discussion

Our analysis indicates that s^2/m relationships are not strongly influenced by either taxonomic specification or environment.

When taxonomic aggregations of major groups (i.e. cladocerans, copepods, rotifers) were examined, we observed s^2/m relationships similar to those found in the Downing et al. (1987), QL, and HR models. Typically, variance of higher order taxa is of a similar magnitude to variance observed at the species and genus level.

We initially postulated that riverine zooplankton might show lower variance because of the physical uniformity of the habitat induced by vigorous mixing. If anything, riverine zooplankton were more variable on average than lacustrine zooplankton based on the differences in intercepts of the QL and HR regression models (Table 1). Downing et al. (1987) also observed that residual variation in their s^2/m relationship was unrelated to environment (freshwater versus marine). On the other hand, s^2/m patterns for benthos differ between streams and lakes/large rivers (Morin 1985). The patterns of variance in plankton as a function of mean abundance appear to be more uniform across environments than is the case for benthos.

Comparison of the HR model with the QL model avoids most of the potential biases of s^2/m comparisons (Downing 1986), as these studies were done using similar sampling gear, similar sampling volume, the same number of replicates, a large number of samples, and a large range of mean abundances. One difference between the studies which could bias the comparison was integrated (QL) versus surface sampling (HR). We cannot, however, assess whether variance might be higher, lower, or equal for integrated samples versus samples from a specific depth.

Taxon-specific regressions were, in general, similar to each other (Table 3). Slopes and intercepts fall within a relatively narrow range. We did, however, find significant differences in the intercepts in three of six cases when comparing s^2/m relationships for taxa common to the HR and QL studies. In these cases, variance was higher in the HR, suggesting that taxon-specific models may not be generalizable to other situations.

The generality of the s^2/m relationships illustrated in Fig. 4 indicate that there are three patterns of spatial distribution of zooplankton at the replicate sample scale. These patterns are inferred from comparisons with a 1:1 line in Fig. 4. If zooplankton were randomly distributed in replicate samples, the s^2/m relationships should conform to a Poisson distribution where the variance equals the mean (Elliot 1977). All three regressions have slopes >1 and cross the 1:1 Poisson line (Fig. 4). Zooplankton, therefore, appear to be more uniformly distributed ($s^2/m < 1$) at low densities (0.01–1 animals·L^{-1}), randomly distributed ($s^2/m = 1$) at moderate densities (1–100 animals·L^{-1}), and aggregated ($s^2/m > 1$) at high densities (>100 animals·L^{-1}).

Population models with random variation in demographic parameters such as population growth and mortality have been demonstrated to yield s^2/m patterns which depend on population density (Anderson et al. 1982). In these models, $s^2 = m$ at low population densities and $s^2 > m$ at medium and high densities. The patterns of uniform distribution ($s^2 < m$) at low densities of zooplankton in the two data sets analyzed in this study and other populations analyzed by Taylor et al. (1983) are inconsistent with predictions of the Anderson et al. (1982) models. These results suggest that s^2/m patterns are not simply a consequence of stochastic population variability through time.

We suggest two possible explanations for zooplankton s^2/m patterns. The spatial distribution of zooplankton at the replicate sample scale may be based on behavior. Taylor and Taylor (1977) have argued that spatial distributions of populations reflect a balance between tendencies of individuals to aggregate versus migrate. Models which simulate these behaviors as a function of population density yield s^2/m patterns consistent with observed data (Taylor and Taylor 1977). A second possibility is that the distributions are a function of sampling. This explanation proposes that the spatial distribution of zooplankton does not vary as a function of abundance, rather the aggregations observed at high density simply become too rare to be sampled at low density. If the second explanation is correct, variance at low and moderate densities should increase as a function of sample volume up to a point because with larger volumes, high-density aggregations are sampled. Unfortunately, we cannot test this idea with our data because replicate sample volume was fixed.

The alternative consequences of our two explanations of spatial distribution are significant. If aggregations of zooplankton change as a function of density, then any spatially dependent effect of zooplankton, for example patchy nutrient regeneration as proposed by Lehman and Scavia (1982), could either be damped (low density) or magnified (high density) across a gradient of zooplankton abundance. Similarly, predators foraging for zooplankton would encounter zooplankton differentially at high and low densities if spatial distribution is changing across a density gradient. On the other hand, if zooplankton spatial distributions are similar at all densities, then spatially dependent effects would be more uniform. An interesting unresolved problem raised by relationships described in this paper is to determine the actual spatial distribution of zooplankton at the scale of sampling.

In conclusion, our analysis supports the approach of Downing et al. (1987). Their model using mean abundance and volume performed well in predicting variance for zooplankton samples drawn from a variety of Quebec lakes, from the Hudson River, and for samples taken in a study of spatial heterogeneity in a specific lake (Pinel-Alloul et al. 1988). The underlying spatial distributions controlling the s^2/m patterns at the replicate sample scale, however, remain unknown. Nevertheless, the regularity of s^2/m relationships enhances testing of hypotheses concerning the influence of ecological factors on zooplankton variability. Sampling variance is predictable, and consequently, confidence in abundance estimates can be controlled by appropriate sampling designs.

Acknowledgements

Financial support was provided by grants from the Hudson River Foundation (Nos. 016/86B/005 and 005/87B/011). We thank S. Baines and H. Cyr for reviewing the manuscript and helpful discussions. This paper is a contribution to the program of the Institute of Ecosystem Studies, New York Botanical Garden, and the McGill Limnology Research Centre.

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Under-Ice Circulation and the Residence Time of a Shallow Bay[1]

Bernard C. Kenney

National Hydrology Research Institute, 11 Innovation Boulevard, Saskatoon, Sask. S7N 3H5, Canada

Kenney, B. C. 1991. Under-ice circulation and the residence time of a shallow bay. Can. J. Fish Aquat. Sci. 48: 152–162.

Vertical temperature profiles measured along the axis of Wupaw Bay in March of three consecutive years showed that the water column was inversely stratified under ice. The depth of the (inverse) thermocline varied monotonically along the length of the bay, with the most rapid change occurring in the channel connecting the bay to Southern Indian Lake. Using prototype instrumentation, the associated two-layer current in the connecting channel was measured. The current near bottom often exceeded 1 cm/s and was directed out of the bay. The upper layer current generally flowed into the bay but was more variable than the bottom current and at times reversed direction. Although the circulation is relatively weak, the water exchange through the channel makes an important contribution to the residence time of water in Wupaw Bay during March. A short (2 h) time series showed that the temperature structure was highly variable below the surface mixed layer. High-frequency random fluctuations in temperature were observed together with active overturning events indicative of significant levels of turbulence under ice. Nonlinear effects also dominated regions with the largest temperature gradients where temperature oscillations appeared wavelike but were higher in frequency than the measured Brunt–Vaisala frequency.

Des profils thermiques verticaux établis le long de l'axe de la baie Wupaw au mois de mars de trois années consécutives ont révélé que la colonne d'eau était stratifiée de façon inverse sous la glace. La profondeur de la thermocline (inverse) variait de façon monotone sur la longueur de la baie; la variation la plus brusque se trouvait dans le canal reliant la baie au lac Southern Indian. Au moyen d'instruments prototypes, le courant à double couche de ce canal a été mesuré. Le courant circulant près du fond avait souvent une vitesse supérieure à 1 cm/s et se dirigeait vers le lac. Le courant de la couche supérieure circulait généralement vers la baie mais était plus variable que le courant de fond et circulait parfois en sens contraire. Bien que la circulation y soit faible, les échanges d'eau par le canal influent de façon importante sur le temps de résidence de l'eau dans la baie Wupaw au mois de mars. Une courte (2 h) série temporelle de données a révélé que la structure thermique sous la couche de mélange de surface était très variable. Des fluctuations de température aléatoires à fréquence élevée ont été observées de même que des renversements des eaux, ce qui témoigne de l'existence d'une turbulence importante sous la glace. Des phénomènes non linéaires caractérisaient aussi les zones montrant les gradients de température les plus élevés et où les fluctuations thermiques suivaient une courbe rappelant celle d'une onde, mais étaient d'une fréquence supérieure à la fréquence de Brunt–Vaisala mesurée.

Received March 20, 1990
Accepted July 23, 1990
(JA514)

Reçu le 20 mars 1990
Accepté le 23 juillet 1990

As part of hydroelectric development in northern Manitoba, the level of southern Indian Lake was raised by 3 m to divert much of the flow of the Churchill River south to power plants on the Nelson River (Newbury et al. 1984). Wupaw Bay, which is connected to Southern Indian Lake by a narrow strait (Fig. 1), has been monitored extensively to determine environmental effects of the increased water level on a typical drainage basin. Both the water mass balance and the chemical budget of the bay were investigated (Hecky et al. 1979).

A primary consideration in determining chemical and nutrient budgets is the residence time of water. Measurements of vertical temperature profiles made along the axis of Wupaw Bay in winter (Hecky et al. 1979) showed that the water column was inversely stratified under ice and that the depth of the interface varied monotonically along the length of the bay. Since a sloping thermocline cannot exist in a static environment, the question arose as to the magnitude of the under-ice circulation and the importance of its contribution to the water residence time.

In this paper, an estimate of the velocity of the under-ice circulation is made using a method devised by Defant (1961) for two-layer channel flow. Results of two techniques to measure slow, under-ice currents are then presented together with measurements of under-ice temperature fluctuations. Relevant literature on buoyancy flux driven circulations is reviewed and possible energy sources for the under-ice flow are discussed. Finally, the potential effect of the circulation on the water residence time of the bay is considered.

Under-ice Temperature Structure in Wupaw Bay

Wupaw Bay extends 12 km south from the lake and is typically 0.5 km wide (Fig. 1). The bay has a mean depth of 5 m compared with 10 m for the adjacent lake. It is joined to the lake by a narrow channel approximately 700 m long, 150 m wide, and 6.5 m deep.

Vertical temperature profiles were taken along the axis of Wupaw Bay at five locations as well as at the experiment site (Fig. 1). Although the ice thickness varied annually from 1981 to 1983, no large variation was seen in the temperature structure (Fig. 2). In each case the vertical profile was stably stratified

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[1] Contribution No. CS-89058.

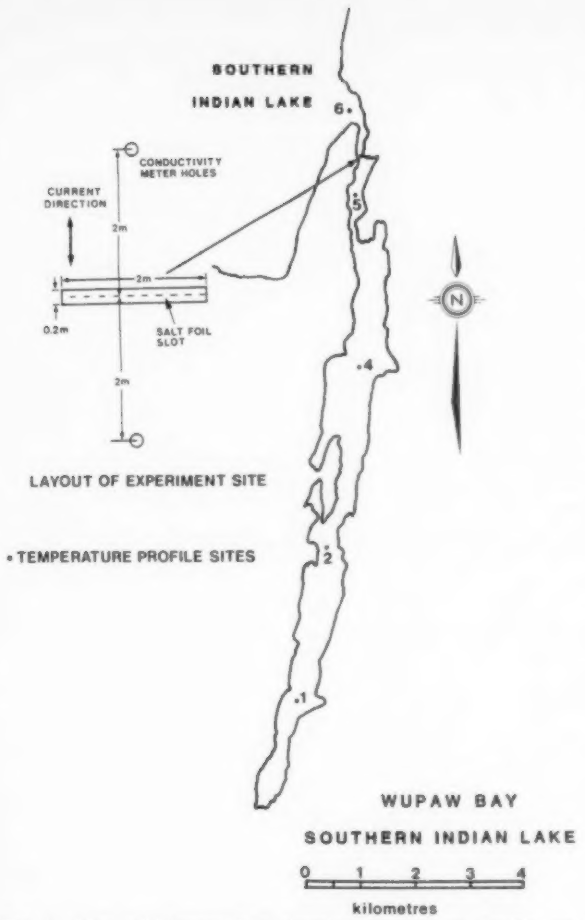


FIG. 1. Map of Wupaw Bay showing temperature profile and conductivity sensor locations.

at temperatures below the 4°C temperature of maximum density of fresh water. The maximum vertical temperature gradient or (inverse) thermocline generally occurred near the 0.5°C isotherm. The depth of the thermocline decreased from the lake towards the head of the bay with the most rapid change occurring in the channel.

A Velocity Scale for the Under-Ice Circulation

The steady sloping thermocline (Fig. 2) is a sufficient condition for the existence of an under-ice baroclinic circulation. The inferred flow is directed out of the bay in the layer below the thermocline and into the bay in the upper layer. A preliminary estimate of the magnitude of the under-ice current in Wupaw Bay (i.e. a velocity scale for the circulation) was obtained using a method described by Defant (1961) for the currents in a narrow strait. To apply Defant's method, I assumed that the under-ice flow was in steady state and that convective accelerations were unimportant. The first assumption will be violated when the baroclinic circulation is first setting up and when (or if) there are rapid changes in the conditions that produce the circulation. The second will be violated wherever there are large changes in the cross-sectional area of Wupaw Bay.

Applying these assumptions, the momentum equation reduces to a balance between the pressure gradient along the bay, dp/dx, produced by the density differences and the resistance to flow, R, caused by friction and turbulence, that is

$$-\frac{1}{\rho}\frac{dp}{dx} - R = 0.$$

The pressure gradient is determined by the slopes of the surface and the density interface and may be estimated with reasonable accuracy from the mean density at each end of the channel (Defant 1961).

The frictional losses are not as well known, particularly for cases of very low flow under ice. Frictional stress, R, is often approximated by $R = k\rho u^2$ where k is the Taylor friction constant and u is the current speed. For a hydraulically smooth channel, k is approximately 0.0025. However, k can be up to two orders of magnitude larger for rough channels. There are also losses that occur due to interfacial stress between the water flowing in opposite directions on either side of the density interface. It is sometimes assumed when constructing hydrodynamic models that the interfacial stress for a summer thermocline is zero but conclusive proof of frictionless flow in the thermocline region is lacking. Virtually nothing is known about possible frictional losses in shear flow across under-ice stratification which is substantially weaker than summer stratification. If it is assumed, however, that the under-ice surface and the lake bottom are both hydraulically smooth and that there are no additional losses by turbulence at the density interface, then an estimate of the magnitude of the under-ice circulation can be obtained that is an upper bound on the baroclinic current when there is equal flow in both layers.

Substituting the quadratic friction equation and the pressure gradient in terms of the mean densities, the mean current in either the upper or lower layer is 1.07 cm/s. This value was taken as an upper limit for the under-ice current to be expected in a smooth channel. If the lake bottom and the under-ice surface are both hydraulically rough, then u could be as low as 0.01 cm/s with the observed horizontal density gradients.

Experimental Methods and Procedure

Wupaw Bay is in a remote area, inaccessible from November to April except by air and snowmobile. One-day experiments to measure the under-ice circulation and temperature profiles were conducted on 17 March 1981, 26 March 1982, and 10 and 11 March 1983.

Thermistor Chain

Ten thermistors, spaced 0.5 m apart, were scanned at 3-s intervals for a period of 1.8 h on 11 March 1983. The overall noise level (in temperature units) calculated from the specifications of the thermistors, voltage regulator, and digital recorder was 0.02°C. The actual noise level in the field was less than the calculated value. All 10 thermistors were calibrated simultaneously in a well-mixed constant-temperature bath against a platinum resistance thermometer with a resolution of 0.01°C. The absolute accuracy of the temperature standard was only ±0.1°C but the accuracy of one thermistor relative to another was determined by the noise level of the individual thermistors.

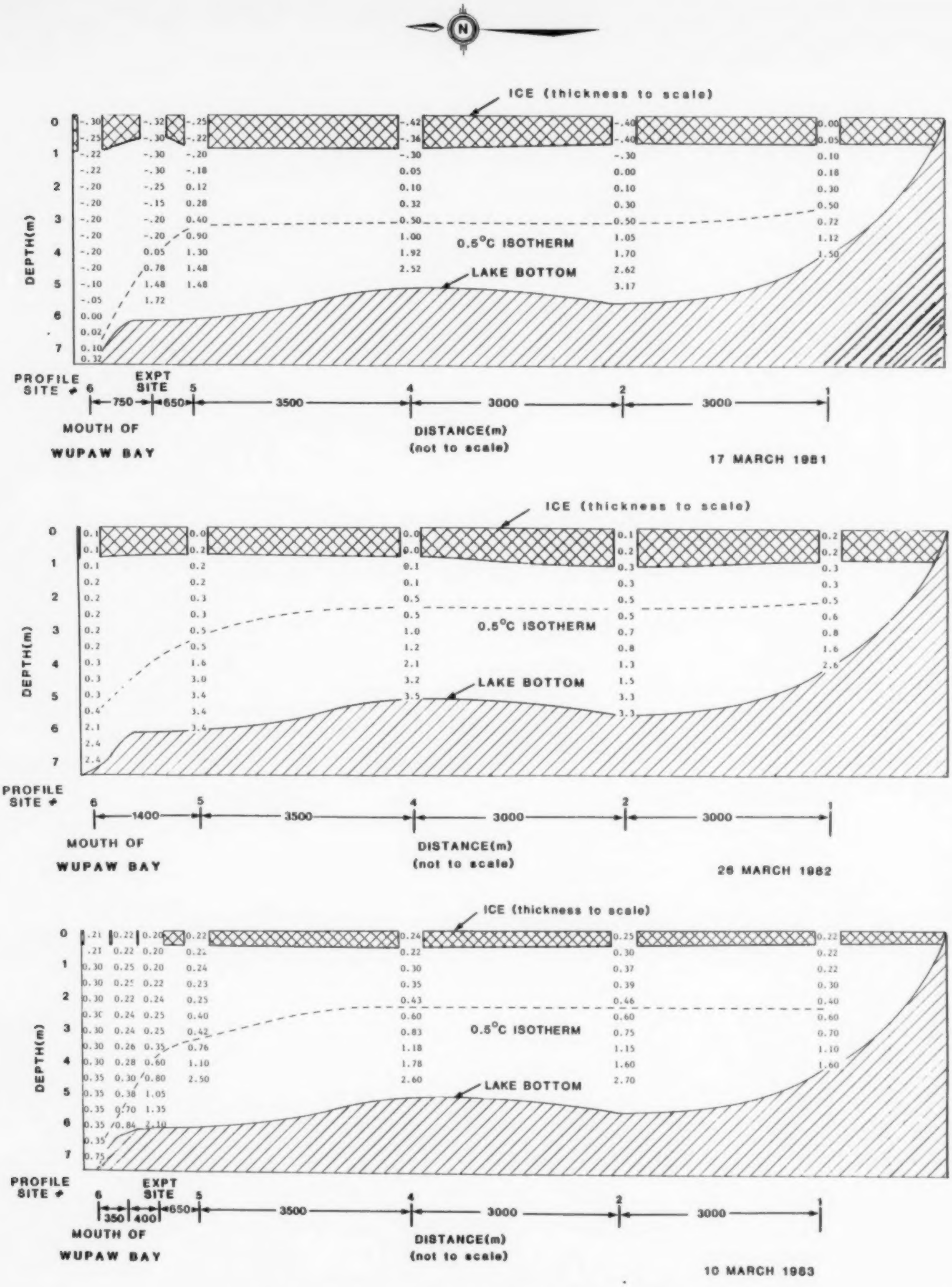


FIG. 2. Temperature profiles (°C) along the axis of Wupaw Bay.

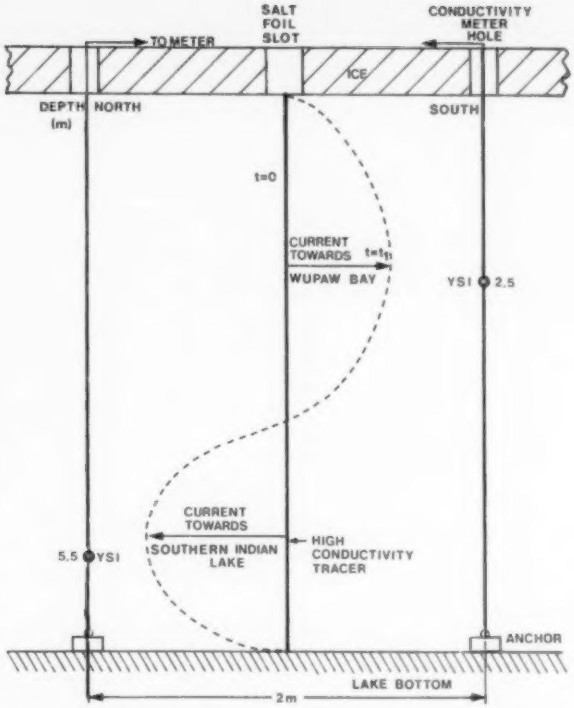
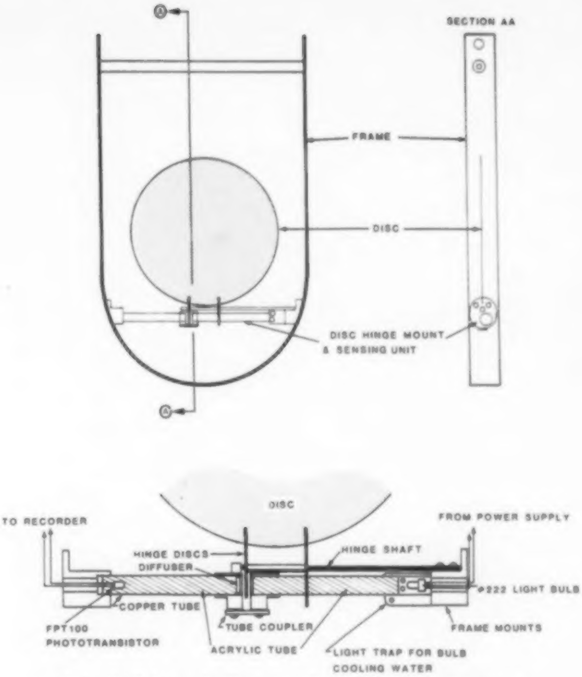

SALT
FOIL
SLOT
CONDUCTIVITY
METER
HOLE
TO METER
ICE
DEPTH
(m)
NORTH
SOUTH
t=0
CURRENT
TOWARDS
WUPAW BAY
t=t1
YSI
2.5
5.5
YSI
CURRENT
TOWARDS
SOUTHERN INDIAN
LAKE
HIGH
CONDUCTIVITY
TRACER
ANCHOR
LAKE BOTTOM
2 m

FIG. 3. Hypothetical two-layer current profile showing a typical layout of the YSI conductivity sensors.


A
A
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& SENSING UNIT
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TO RECORDER
FROM POWER SUPPLY
HINGE DISCS
DIFFUSER
HINGE SHAFT
#222 LIGHT BULB
COPPER TUBE
FPT100
PHOTOTRANSISTOR
TUBE COUPLER
ACRYLIC TUBE
LIGHT TRAP FOR BULB
COOLING WATER
FRAME MOUNTS

FIG. 4. Schematic diagram of drag disk current meter.

Conductivity Tracer

Two YSI conductivity sensors were installed 2 m from a central slot (Fig. 1) to determine the mean current at two depths in the channel from the time of travel of a conductivity tracer introduced in the slot. Figure 3 shows the vertical locations of the sensors relative to a (hypothetical) conductivity profile produced by a two-layer flow. The output of the YSI meters and the precise time of each reading were recorded manually.

The conductivity tracer was generated as a line source in the wake of a streamlined aerofoil coated with crystals of NaCl and KCl. The salt mixture consisted of 20% fine-grained KCl, 50% NaCl in table salt form, and 30% NaCl as coarse pickling salt. The KCl dissoled quickly in the top portion of the water column, while the NaCl ensured that salt was available for marking the centre and the bottom portion of the water column.

To minimize the disturbance to the water column, the source was lowered at a uniform rate of about 0.2 m/s using monofilament lines attached to the trailing edge of the aerofoil. The conductivity increase was large enough to be detectable by the sensors (~50 μS/cm) but not so large as to be dynamically important. Tests made in a laboratory tank filled with still water at 4°C showed a narrow wake with reasonably uniform conductivity behind the source.

Drag Disk Current Meter

A prototype current meter was developed to measure under-ice currents in the range from 0.1 to 1.0 cm/s (Fig. 4). The value of 1.0 cm/s was picked as the upper design limit for the current meter from the results of applying Defant's (1961) method to a hydraulically smooth channel. The current meter was designed to be quick and simple to operate at −40°C and to fit in a standard briefcase to facilitate use from aircraft.

The current sensor was a flat high-density polyethylene disk 18.97 cm in diameter and 0.914 mm thick with a specific gravity of 0.996. When hinged to a supporting framework by two smaller disks, the drag disk floated vertically in still water but was very sensitive to rotation by currents normal to it. One of the small hinge disks was also used to modulate the light supplied to a phototransistor as the drag disk turned. Both hinge disks were statically balanced and the hinge bearings were milled to a knife edge to reduce friction with the shaft.

The sensitivity of the current meter was calculated from laboratory measurements. First, the relationship between the angle of deflection of the drag disk and the output voltage was determined empirically by rotating the meter on a turntable submerged in still, 4°C water in a laboratory tank. Then, the relationship between the angle of deflection and the current speed was calculated by balancing moments produced by the buoyancy force of the disk and its drag, using the known drag coefficient of a circular disk as a function of Reynolds number. Friction in the drag disk pivots was neglected because there was no detectable hysteresis during the angle-voltage measurements. The buoyancy of the disk was directly affected by volumetric changes caused by thermal expansion or contraction (1.3×10^{-4}/°C). Although the effect of thermal expansion was small over the range of under-ice temperatures in Wupaw Bay, it was incuded in the calculated sensitivity of the current meter.

No low-speed test facilities were available to calibrate the meter directly. Moving the meter through still water at nearly constant speed over a distance of 10 cm in 10 s, however, demonstrated that the calculated sensitivity was approximately correct in a large laboratory tank.

The drag disk and its associated detection system was mounted in a U-shaped flat steel frame designed to minimize the total drag on the meter. The current meter was suspended by steel cables from a leveling frame mounted over a hole in the ice. The hole was then covered with a black plastic sheet to shade the water column from direct solar radiation. The weight of the current meter (1.25 kg) was sufficient to permit the current meter to be suspended from cables without danger of oscillating in the low speed currents. The current was measured at 1-m intrevals by lowering the current meter on its suspension cables. Conditions were allowed to stabilize for 5 min at each depth before recording the output on a data logger.

Results and Discussion

Time of Travel of the Conductivity Tracer

The conductivity experiments were designed with the assumption that the under-ice turbulence levels were low. The observed conductivity increase (Fig. 5) was smaller under-ice in Wupaw Bay, however, than in the laboratory. The wake was also much wider than expected from the laboratory tests. Both effects were attributed to unexpectedly high levels of turbulent mixing.

Calculated from the time of travel of the peak concentration detected by the YSI sensors, the current speed was similar to the velocity scale calculated using Defant's (1961) method. There was considerable variation in speed, however, from one run to another (1 cm/s ± 30%).

Drag Disk Current Meter

On 10 March 1983, the drag disk was deployed consecutively at five depths (Fig. 6). The actual current speed frequently exceeded the 1 cm/s design criterion and the disk was forced against mechanical stops. Although the current in the upper half of the water column was generally into the bay, numerous fluctuations (8- to 10-min period) and even unexpected reversals in the current direction were observed. Near bottom, the current was directed out of the bay as expected from the horizontal density gradients along the axis of the bay.

When the current was within the design limits of the meter, it was not steady. In addition to the 8- to 10-min fluctuations in current magnitude and direction, the natural frequency of the drag disk was often excited by turbulence and/or internal waves. Intense, short-period oscillations (Fig. 6) occurred very near to the 37.5 ± 2.5 s damped natural period of the drag disk (measured in still water). A subcritical damping factor of 0.28 was measured in the labortory for the disk which allows for a resonant response of almost 200% for forcing near the natural frequency of the disk. The low-frequency response of the current meter was not affected by resonance.

Under-Ice Temperature Profiles

All the vertical temperature profiles were statically stable (Fig. 7). The first 3.9 m below the surface was reasonably well mixed, however, with the temperature increasing only slightly from 0.1 to 0.3°C. Below 3.9 m, the vertical temperature structure changed rapidly during the course of the experiment. Initially, a well-mixed region (at about 1.5°C) was observed between 4.9 and 5.9 m. This region was unusual because it occurred in the middle of the water column and was delimited above and below by sharp temperature gradients (2°C/m). At 16:50 CST (just past the midpoint in Fig. 7), the 1.5°C water was replaced rather suddenly with water with a significant vertical temperature gradient. At the same time, a homogeneous layer of 2.4°C water apeared near the bottom. These latter profiles were closer to the expected form for the vertical temperature profile in a two-layer flow (i.e. homogeneous surface and bottom layers separated by a strong temperature gradient).

Under-Ice Current and Temperature Fluctuations

The rapid change in the thermal structure that began about 16:50 was accompanied by oscillations in the current (Fig. 8). Prior to 16:50 the current at 4.4 m exceeded the limit of the

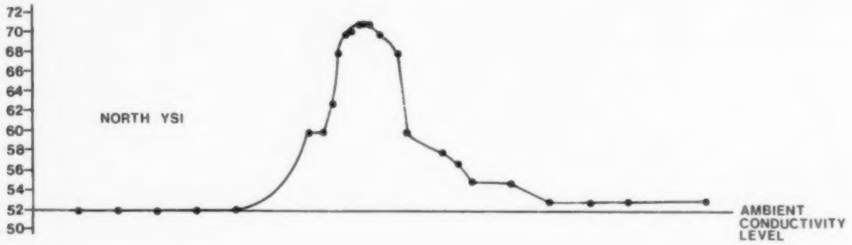

FIG. 5. Temporal response of two YSI sensors to a line source of conductivity, 17 March 1981. The south sensor was at a depth of 2.5 m and the north sensor was at a depth of 5.5 m.

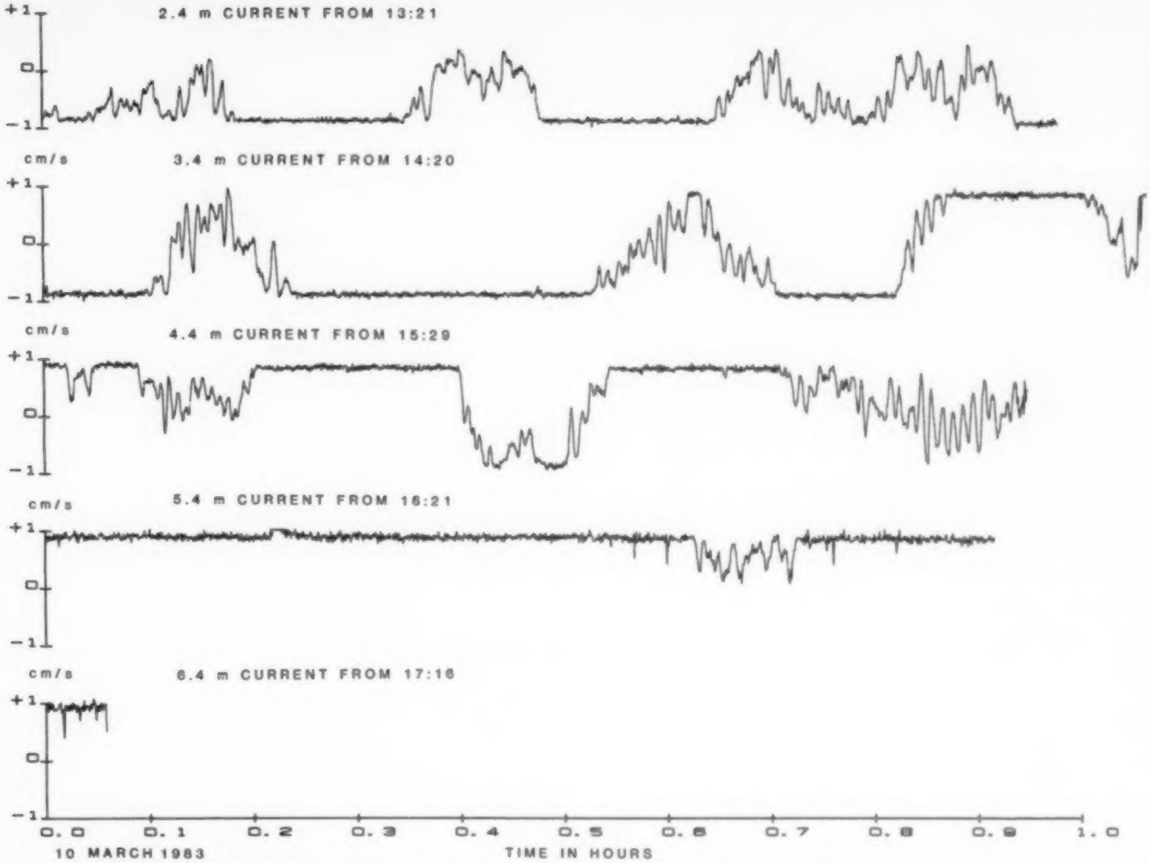

FIG. 6. Current speed measured on 10 March 1983 at various depths in the channel connecting Wupaw Bay and Southern Indian Lake. A positive current is directed out of Wupaw Bay. The current meter output had hard limits at ± 1 cm/s.

meter and was directed out of the bay. The current meter remained saturated until about 16:50 when an oscillation in current accompanied changes in the temperature. The flow at 4.4 m reversed direction for almost 4 min during this 10-min oscillation.

Although isolated from the usual wind-driven sources of turbulence at the surface by 0.6 m of ice, the time histories of temperature (Fig. 8) clearly illustrate the dynamic nature of thermal structure under ice. Frequent temperature inversions occurred in the top 4 m that suggest the presence of active or incipient mixing. After 17:00, inversions were also observed near bottom accompanied by inversely correlated, high-frequency (T = 0.5 min) oscillations, suggestive of active overturning (Fig. 9).

The mixed region of 1.5°C water was dominated by random temperature fluctuations and may have been produced by turbulence generated by shear between the inflow and the outflow in the channel. With the observed temperature gradients, the shear required to sustain mixing (i.e. to bring the gradient Richardson number to below 0.25) is 0.04/s. This level of shear may be reached with the observed currents by a linear shear between the upper and lower flows that is <0.5 m thick. Although the current meter results give some direct evidence of a two-layer flow in the channel, the thickness of the shear between the two layers was not established. The mean temperature profiles (Fig. 7) indicate that the shear layer between inflow and outflow may have been closer to 2 m thick.

The largest temperature fluctuations were measured in the regions where temperature gradients were the highest. These large oscillations were more periodic and indicate the presence of internal wave motions. It is unlikely that these large oscillations were produced by simple linear internal waves, however, because their frequency exceeded the local Brunt–Vasaila frequency at those depths. For example, beginning at 16:30, here was an abrupt rise in temperature at 5.9 m followed immediately by several large oscillations with a period of about 2.2 min. These oscillations were considerably shorter than the minimum observed value of local Brunt–Vasaila period of about 4 min. The short period may have resulted from a Doppler shift caused by the current. It is also possible that the internal waves were strongly influenced by nonlinear effects that produced significant wave steepening. No temperature inversions were associated with any of these large periodic oscillations, however, that would suggest actual internal wave breaking or turbulent overturning.

It is not immediately obvious what is causing such dramatic oscillations in flow and temperature and there appears to be little assistance in the literature. The variation in temperature at 5.9 m, beginning at 16:30, had some resemblance to an internal surge or bore such as those reported by Ziegenbein (1969, 1970) and Hunkins and Fliegel (1973). The signature of an

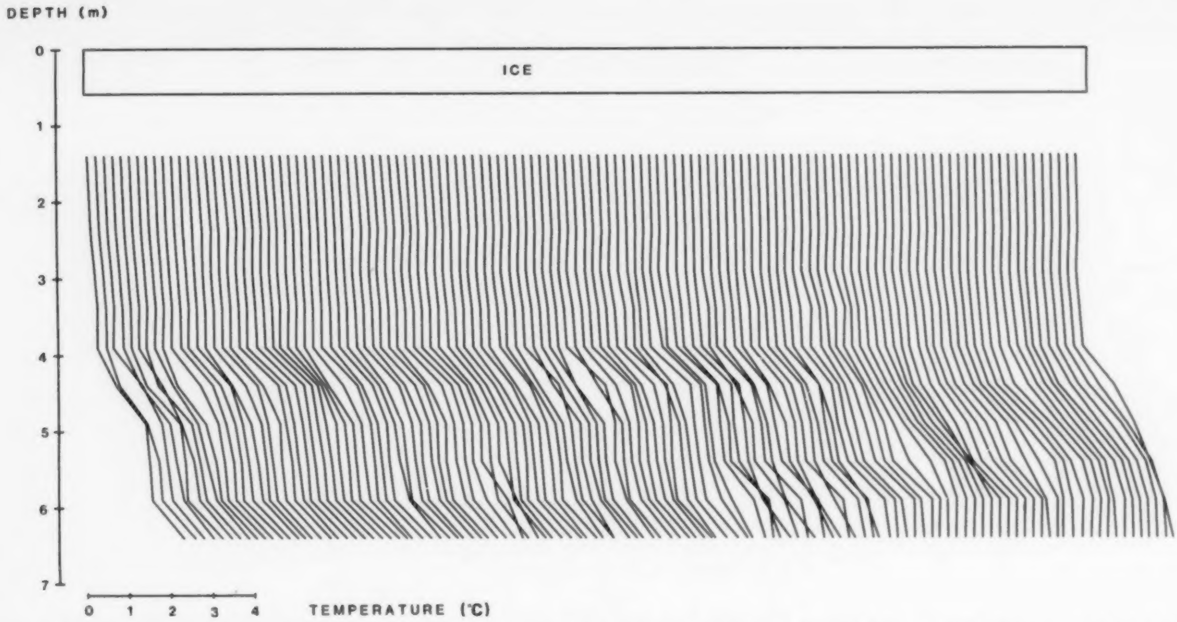

FIG. 7. Vertical profiles of temperature (averaged over 54-s intervals) from 15:30 to 17:18 CST, 11 March 1983. Each successive profile is plotted 0.2°C to the right of the previous profile.

internal bore is a sharp increase in temperature at fixed depth followed by a series of high-frequency oscillations (periods vary from 6 to 20 min) that terminates with a steady elevated temperature. While the shape of the temperature fluctuations at 5.9 m is somewhat similar to reported bores, there are two important differences. Firstly, the density stratification is the inverse of the temperature stratification under ice so that the energy condition for a bore is not met (i.e. the potential energy of the water column must increase following the passage of a bore). Secondly, the high-frequency oscillations that followed the sharp temperature increase at 6.9 m were much higher than with other reported bores and exceeded the local Brunt–Vasaila frequency.

Although the exact cause of the observed temperature and current oscillations is uncertain, it is clear from these measurements that nonlinear effects strongly influence both current and temperature fluctuations under ice.

Potential Energy Sources for Under-Ice Circulation

Buoyancy Flux Driven Circulation

The under-ice circulation can be treated as a buoyancy flux driven circulation where the horizontal buoyancy flux through the channel is balanced against the net vertical buoyancy fluxes through the surface and bottom of the bay.

The total buoyancy flux, B_A, associated with the under-ice circulation between Wupaw Bay and the lake can be estimated from

$$B_A = \int Ub\,dA$$

where U is the flow velocity at a point in the cross-sectional area of the channel, dA, and b is the buoyancy of an elemental parcel of water. The buoyancy is defined by its density, ρ, relative to the reference density, ρ_0, by

$$b = -g(\rho - \rho_0)/\rho_0 .$$

Using a value for b calculated from a typical temperature profile and the order of magnitude estimate for U of 1 cm/s:

$$B_A = 1.6 \times 10^{-3}\ \mathrm{m^4/s} .$$

The buoyancy flux through the channel is directed into the bay from the lake. Since buoyancy is a conserved quantity in the circulation, the net buoyancy flux through the channel into Wupaw Bay must be balanced by a net buoyancy flux out of the bay, either upwards through the surface or downwards through the bottom of the bay or both.

Following Philips (1966), the surface buoyancy flux, B_0, can be produced either by sensible (or radiative) heat transfer, Q_s, by evaporation, E, or by evaporation increasing the salinity, S. If g is the acceleration of gravity, ρ_0 is the reference density, L is the latent heat of evaporation, α_v is coefficient of thermal expansion, and C_p is the specific heat of water at constant pressure, the buoyancy flux can be described by

$$B_0 = -\frac{g}{\rho_o}\left[(Q_s - LE)\frac{\alpha_v}{C_p} - ES\right].$$

In the present case, both the evaporation and salt concentration terms drop out. Furthermore, the under-ice water temperature of the bay is less than 4°C and α_v is negative. Therefore, the buoyancy flux resulting from the sensible heat transfer through the surface is directed downwards. That is, the surface waters are becoming more buoyant as a result of the surface cooling. Any solar radiation that penetrates the snow and ice, however, produces a loss of buoyancy near the surface. The net buoyancy flux at the water surface is dependent upon the relative magnitudes of these effects.

There is also a buoyancy flux at the sediment–water interface during winter produced by the release of heat stored in the

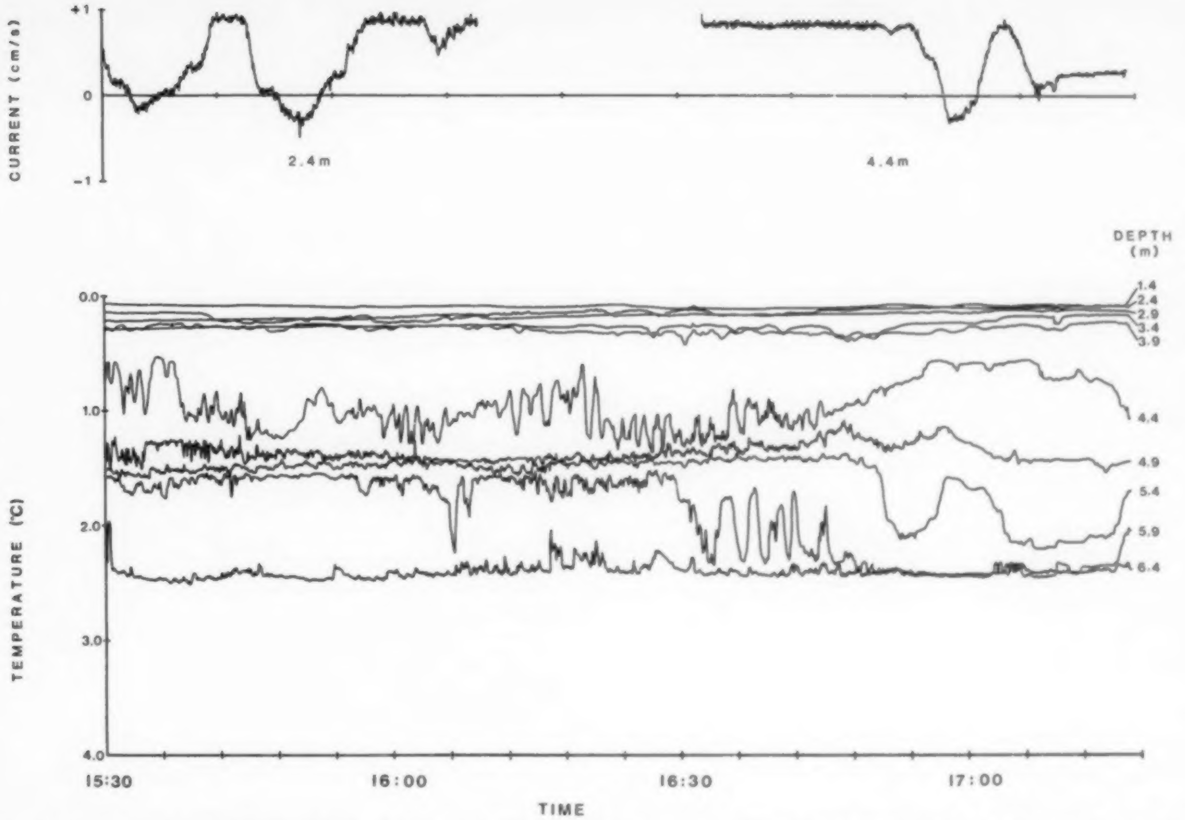

FIG. 8. Current speed and temperature fluctuations in the Channel connecting Wupaw Bay and Southern Indian Lake, 11 March 1983. A positive current is directed out of Wupaw Bay. The current meter output had hard limits at ± 1 cm/s.

sediment. The buoyancy flux at the bottom is directed downwards out of the water and into the sediment. The bottom waters are becoming less buoyant as a result of this heat exchange.

The value of B_A calculated above is now compared with estimates of the buoyancy fluxes through the bottom and surface areas of the bay.

Heat and Buoyancy Fluxes at the Bottom of Wupaw Bay

The annual heat exchange between the sediments and the bottom waters can be estimated using the one-dimensional diffusion equation

$$(1)\quad \frac{dT}{dt} = D\frac{d^2T}{dz^2}$$

where the lake bottom is assumed to be the surface of a semi-infinite solid. The temperature, T, at any depth, z, in the sediment changes with time, t, and depends upon the diffusivity, D. The boundary condition at the lake bottom during the heat flow is the fluctuating temperature of the bottom water which can be approximated as an annual oscillation of the form

$$T(0, t) = T_0 \sin 2\pi ft$$

where T_0 is the amplitude and f is the frequency of oscillation (taken as 1 cycle/yr). It is also assumed that the temperature remains finite as the depth approaches infinity, that is, $T < \infty$, $z \rightarrow \infty$.

Under these conditions, the solution of equation 1 is

$$(2)\quad T(z, t) = T_0 \exp\left(-z\sqrt{\pi f/D}\right) \sin\left(2\pi ft - z\sqrt{\pi f/D}\right)$$

which shows that the temperature at any depth, z, in the sediment fluctuates at the same frequency as the lake bottom temperature but the amplitude of the temperature fluctuations decrease exponentially with depth. Equation 2 also shows that the time lag between the interface temperature and the temperature at depth z increases with increasing depth in the sediment.

The instantaneous heat flow rate at the lake bottom may be found from the temperature gradient at the sediment–water interface using the flux-gradient equation, that is

$$(3)\quad (q/A) = -\rho_s C_{ps} D(dT/dz)_{z=0}$$

where q/A is the heat flux or instantaneous heat flow rate per unit surface area, ρ_s is the density of the sediment, C_{ps} is the specific heat of the sediment, and dT/dz is the vertical temperature gradient.

Differentiating equation 2 with respect to z, substituting the result into equation 2, and simplifying produces an equation for the heat flux:

$$(4)\quad q/A = \rho C_p D T_0 \sqrt{2\pi f/D}\, \sin\left(2\pi ft + \frac{\pi}{4}\right).$$

In order to apply the results of the above analysis to Wupaw Bay, the temperature of the bottom water and the density, spe-

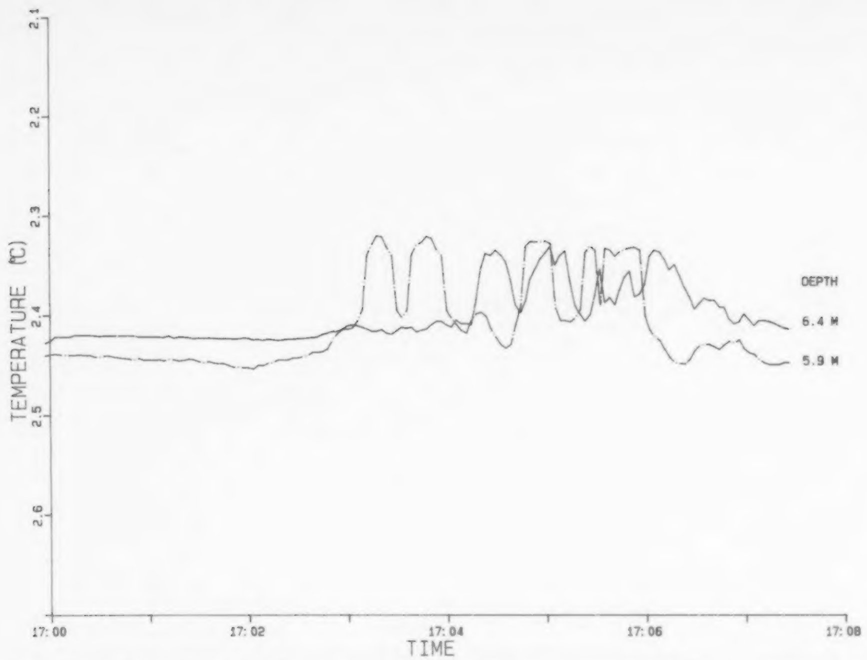

FIG. 9. Time history of temperature (expanded from Fig. 8) showng near-bottom inversions and inversely correlated oscillations that suggest convective overturning.

cific heat, and thermal diffusivity of the sediments must be known. No data are available on the pertinent characteristics of the sediments, however, so typical values were taken from the literature.

Several years of data are available for the bottom water temperature (Hecky et al. 1979) which allows one to estimate the amplitude of the annual oscillation of the bottom temperature. In some early limnological work on Lake Mendota (Birge et al. 1928), a value for thermal diffusivity, D, of 0.00325 cm^2/s was obtained. Neumann (1953) reported a slightly higher value of $D = 0.004$. In both studies it was assumed that the product $\rho_s C_{ps} = 1$. Using $D = 0.004$ cm^2/s as typical of the thermal diffusivity of Wupaw Bay and $T_0 =$ 8°C from Hecky et al. (1979), the maximum heat flow out of the sediments and into the bay is 2.26×10^{-4} cal·cm^{-2}·s^{-1} (1 cal = 4.1868 J). This maximum heat flux occurs in mid-December and produces an associated buoyancy flux out of the bay of $B_s = 7.3 \times 10^{-3}$ m^{4}s^3.

The total buoyancy flux through the sediment–water interface as calculated for mid-December is 4 times that required to drive the circulation through the channel between Wupaw Bay and the lake. The present observations were made in mid-March, however, and by that time the heat flux out of the sediments (and concomitant buoyancy flux) has fallen to zero. Therefore, while the heat stored in the sediment may be large enough to drive a circulation in December, it cannot be the energy source for the March circulation.

Heat and Buoyancy Fluxes at the Surface of Wupaw Bay

The sensible and radiative heat fluxes are in opposite directions at the ice–water interface. As shown below, the positive buoyancy flux associated with the sensible heat loss from the water column subtracts from that necessary to drive the observed circulation with inflow at the surface and outflow at the bottom of the channel. The short-wave radiative flux, on the other hand, represents a strong negative buoyancy source for such circulations.

Sensible heat flux

A shallow, wind-mixed lake contains little excess temperature (and very little heat) above the freezing point of water at the initiation of freeze-up. For example, the excess heat content of Wupaw Bay which has a mean depth of 4.5 m and mean temperature of about 2°C at freeze-up is typically 900 cal/cm^2 (relative to 0°C). In spite of air temperatures as low as −40°C from November to March, the heat content of the water column does not decrease much after freeze-up. Once ice formation decouples the water column from the wind, the major heat source for the sensible heat transfer upwards through about 1.0 m of ice is the latent heat of fusion at the ice–water interface. Since 900 cal/cm^2 is equivalent to the latent heat required to freeze only 11 cm of ice, it is clear that the water column itself cannot be a major heat source for the upwards heat flux. The main effect of the −40°C temperatures, therefore, is to thicken the ice layer. During those winters when deep snow covers the ice, the snow acts as insulation, greatly reducing the sensible heat loss and the rate of thickening of the ice.

It is the heat flux out of the water column, however, that is relevant to the under-ice circulation. Our measurements show that the temperature gradient directly below the ice is small. Moreover, upwards heat flux tends to stabilize the water column in Wupaw Bay and suppress turbulence and convective currents that produce heat transfer. Although the heat loss from the water column produced by low air temperatures appears small, an accurate estimate of the heat flux is not possible with available data.

Short-wave radiative flux

The undepleted insolation in mid-March at the latitude of Wupaw Bay is 500 $\mathrm{cal \cdot cm^{-2} \cdot d^{-1}}$. The buoyancy flux through the channel into the bay, however, is equivalent to that produced by only 1% of the incident solar radiation. Thus, penetration of solar radiation is a potentially powerful source of energy to counter the sensible heat loss through the ice and drive an under-ice circulation in mid-March.

The amount of solar energy that actually penetrates the ice and snow cover is a small fraction of the incident radiation. Much of the light is reflected. The total albedo can be as high as 0.9 for new snow but decreases as the snow corns with age, i.e. forms large rough crystals by sublimation over time (Bruce and Clark 1966). On clear days, the albedo is lower than when the sky is overcast. Typical values are 0.6 and 0.7, respectively.

Light is also strongly attenuated in snow. Curl et al. (1972) measured the attenuation of spectral intensity in a snowpack similar to that covering Wupaw Bay in March 1983. The snow had a hard crust of old crystalline snow that had corned on the surface. From data collected at six times throughout the year, the attenuation of total incident solar radiation, A (percent), with depth in snow, D (centimetres), followed the power law equation $A = 104.5\,D^{-1.39}$. The attenuation through 50 cm of snow, however, varied from 2 to 0.2% from one data set to another. Light attenuation can also be significant through white ice and slush. The attentuation through black ice is similar to that of water (H. Welch, Freshwater Institute, Winnipeg, Man., pers. comm.).

Wupaw Bay was covered by a blanket of snow overlying up to a metre of ice. In 1983, the snow was about 50 cm deep in the channel between the bay and the lake. There was considerable variation in the thickness of the snow, both spatially throughout the bay and from year to year. In wind-swept areas, the snow was often less than 20 cm deep. The ice layer consisted of mostly black ice but varying amounts of white ice and/or slush were present at the snow–ice interface. Although sufficient data on the temporal and spatial variation of snow and ice types, thicknesses, and properties are not available to warrant a calculation of the average radiative flux into the water column, light penetration through the ice and snow represents the most probable energy source for a March circulation. Although detailed temperature surveys were not made in December, there is sufficient heat stored in the sediments to drive an under-ice circulation in early winter.

Importance of Under-Ice Circulation to Water Residence Time

The water residence time is a key parameter determining the dynamic response of lakes and reservoirs to changes in nutrient inputs (Kenney 1990). From measured values of local runoff from the drainage basin, the average water residence time of the bay is 20 yr (R. E. Hecky, Freshwater Institute, Winnipeg, Man., pers. comm.). Most runoff occurs in the spring, however, so that the annual average water residence time may be inadequate for specifying the dynamic response of the bay over much of the year. The use of a time series of instantaneous water residence times (lake volume/total inflow at any instant in time) is more appropriate for dynamic environments with large changes in inflow throughout the year (Kenney 1990).

When runoff alone is considered, the instantaneous water residence time is infinite in winter because there are no rivers flowing into the bay and the local streams are frozen. Groundwater inflow is also assumed to be small because Wupaw Bay is located in a permafrost zone. Therefore, the under-ice circulation will directly determine the instantaneous water residence time during the 6-mo ice-covered period.

The water residence time when the bay is covered with ice can be estimated if the circulation in the channel is considered to be a two-layer flow that persists for 6 mo. Assuming, as a first approximation, equal flow in both layers and a velocity scale of 1 cm/s, the water residence time based solely on the under-ice circulation is 0.67 yr. Since this value is very short compared with the average water residence time due to annual runoff, the under-ice circulation may also control the dynamic response of Wupaw Bay to changes in chemical and nutrient inputs for much of the year.

In conclusion, prototype instrumentation was used to measure under-ice currents and temperature at various depths in the channel connecting Wupaw Bay to Southern Indian Lake. The vertical temperature structure was found to change rapidly under ice, possibly a result of an internal surge. Strong nonlinearities were evident in the temperature signals. Temperature fluctuations at various depths were often wavelike in appearance but higher in frequency than the measured Brunt–Vaisala frequency. Temperature inversions and evidence of active overturning in mixed regions also suggest the presence of significant levels of turbulence under ice.

The under-ice circulation was directed out of the bay near the bottom and generally into the bay near the surface. The upper layer currents were unsteady and often changed direction. The current speed in each layer often exceeded 1 cm/s. The circulation occurs annually in March and may exist all winter. Possible energy sources are heat flow from the sediments in early winter and penetration of solar radiation through the ice in the spring. The under-ice circulation appears important to the water residence time of Wupaw Bay even though the velocities associated with the circulation are small. Similar buoyancy-driven circulation probably exists under ice in other enclosed bays provided that the depth of the bay differs significantly from that of the connecting lake. The relative importance of the under-ice circulation to the water residence time of a bay depends upon the existence and the magnitude of any riverine flow through that bay in winter.

Acknowledgements

The author is indebted to Dr. R. Hecky, Department of Fisheries and Oceans, for arranging financial and logistical support for several field experiments. J. Mollison constructed the instrumentation and with J. Tisdale contributed much to the field work. M. Isaac assisted with the computer analysis. M. Waiser and an anonymous reviewer provided comments that improved the clarity of the manuscript.

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Guide for Authors

Introduction

We welcome manuscripts reporting significant new information in fisheries and aquatic sciences. Manuscripts may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and cover numerous disciplines including biology and ecology of marine and freshwater organisms, physiology, toxicology, genetics, limnology, oceanography, economics, disease, and management.

Manuscripts are selected for publication according to the extent and significance of new knowledge or ideas presented. Preference will be given to those whose experimental results emphasize causal mechanisms underlying observed phenomena rather than pure description and fact. Manuscripts should lead to identifiable conclusions or synthesis, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. Their novelty must relate to more than the particular (a certain year, place, taxon, chemical compound) and their contribution to science must clearly be beyond the confirmatory state.

Manuscripts submitted should be as comprehensive as possible; research fragments are generally unacceptable. If a single paper cannot be produced, then closely related papers should be cross-referenced and submitted together. The paper should not be submitted or accepted for publication elsewhere.

The *Journal* considers the following types of contributions:

Articles: Studies of broad scope that are original contributions to science.

Reviews: Detailed critical appraisals of broad areas of investigation, particularly those that have developed rapidly in recent years.

Perspectives: Essays of opinion or hypothesis on aquatic science of concern to the professional and layperson. References are desirable; informal style is welcome.

Comments: Opinion on topics recently discussed in the *Journal*; preferred maximum length, 500 words.

Manuscript Submission

Submit manuscripts in triplicate to the Editor, accompanied by a covering letter that (1) states the main points and significance of the work, (2) accepts the mandatory $44 page charge, (3) suggests potential referees, and (4) identifies other manuscripts containing the same or similar information. Original typescripts and figures (except halftones) are best retained by the author until requested.

Each manuscript is normally submitted to two referees for appraisal before final evaluation by an editor. However, the Editor will return unreviewed those manuscripts that do not fall within the *Journal's* editorial policy or scope.

Editorial Practices

The *Council of Biology Editors Style Manual* (5th ed. CBE, 9650 Rockville Pike, Bethesda, MD 20814) is used as the authority in matters of form. The standards for spelling are either *Webster's Third International Dictionary* or the *Oxford English Dictionary* and *Le Grand Larousse encyclopédique*. Authors are responsible for consistency in spelling. Titles of periodicals are abbreviated as in *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103). Authors are responsible for ensuring the accuracy and completeness of their reference list. The *Journal* follows the names and spelling for fishes recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (4th ed. 1980. Spec. Publ. No. 12, American Fisheries Society) and the gene nomenclature for protein-coding loci outlined in Shaklee et al. (1990. Trans. Am. Fish. Soc. 119: 2–15). All measurements must be metric, use SI symbols in most instances (exceptions noted in editorial in the *Journal*, Vol. 40, No. 12), and adhere to the *Canadian Metric Practice Guide* CAN3-Z234.1-79 (Canadian Standards Association, 178 Rexdale Boulevard, Rexdale, Ont. M9W 1R3).

Series titles should be avoided. An abstract is required for all manuscripts and should state concisely, in up to 175 words, what was done, found, and concluded.

Proofs are sent to the author, who is responsible for correcting errors in typesetting. *Alterations to the content must be avoided.*

Fifty reprints are supplied free. Additional reprints may be purchased at the time of publication (an order form is sent with the proofs).

Preparation of Manuscripts

General Guidelines

Movement of manuscripts through review channels and the editorial office is greatly expedited if manuscripts are prepared in *Journal* style and format. When in doubt about specific details, consult a current issue of the *Journal*. General guidelines follow.

Type the manuscript on white paper, preferably 21.5 × 28 cm (8.5 × 11 in.), on only one side of the page. Leave all margins at least 2.5 cm wide.

Type only the title, authors' names and affiliations, and related footnotes on the first page. Number all pages beginning with the title page, including those for tables and captions for illustrations.

Double-space all parts of the manuscript, including tables, captions for tables and figures, footnotes, and the reference list. Underline only material that is to be printed in italics.

Do not use all capitals anywhere in the manuscript.

Use the name-and-year system for literature citations.

Checklist for Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables and figures and the text, or within the text? Should some of the data be made available separately in a data or manuscript report or at a data repository?

If statistical analysis is included, is it subordinate to the research? When probability statements are made, are only the statistical tests cited and unnecessary statistical tables excluded?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report? Does the *abstract* give the essentials of the new knowledge? Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of *materials and methods* limited to what scientists need to understand the design of the study and to judge the adequacy of the data? Are generalizations from the *results* supported by the data provided? Are findings distinguished from inferences? Is the *discussion* limited to interpretation and significance of the findings?

The most common technical problems in submitted manuscripts are listed below. Authors can hasten the processing of their papers if they pay attention to these points during manuscript preparation.

1) Insert bibliographic strip before the abstract (Authors, date, title, Can. J. Fish. Aquat. Sci.).

2) Limit abstract to one paragraph of about 175 words.

3) Double-space all elements of the manuscript, including references, table captions, and figure legends.

4) Avoid exclusive use of capitals everywhere in the manuscript, including headings, table captions, and figure legends.

5) Underline only Latin names of organisms and certain statistical and mathematical notation to indicate italics.

6) Use correct SI symbols for units or measure in figures, tables, and text. Place a zero before the decimal for numbers less than unity.

7) Avoid ambiguous forms such as $g\ C/m^2/d$; use $g\ C{\cdot}m^{-2}{\cdot}d^{-1}$.

8) Identify test used to test statistical significance and give probability value. No reference is needed for common statistical tests.

9) In the text, generalize from tables and figures; avoid repeating all the details. Be sure each table and figure can stand on its own and is referred to in the text in numerical order. The captions should explain the purpose of the table or figure.

10) Include acknowledgments section at manuscript stage, not when galley proofs arrive.

11) Provide the person's initials and mailing address when referring to personal communications.

12) Delete unnecessary references that do not apply directly to the problem.

13) Check references carefully against test citations and vice versa to ensure exact correspondence. Provide an availability statement for less easily retrieved material, e.g. available from Department of Economics, Simon Fraser University, Burnaby, B.C. V5A 1S6.

14) Delete commas between name and date in citations such as (Smith 1900) and do not underline "et al."

15) Replace 10^n in table headings and figure axes with appropriate SI prefixes or use words (e.g. thousands).

16) Photocopies of figures, except halftones, are appropriate for review purposes. Send photographic prints of figures, or originals if they do not exceed 21.5 × 28 cm, on request for publication. Use the same type of lettering throughout if possible.

Parts of the Manuscript

Organization

Organize the manuscript on the basis of the purpose or scope of the study as stated in the introduction. Ensure that the title and headings are in harmony with the statement of purpose.

Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

Organize tables and figures to facilitate comparisons, grouping related data in as few tables and figures as feasible. As far as possible, make the tables and figures clear without reference to the text.

Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

See that everything in each section is relevant to the heading, and everything in each paragraph to the topic (opening) sentence.

Before writing any paragraphs, try writing the topic sentences for all of them and arranging these in appropriate order.

Title

Limit the title to what is documented in the manuscript. It is the key to the article and should clearly and concisely reveal what appears in the paper itself. The title serves two functions: (1) it allows the reader to judge whether or not the article is of potential interest and (2) it should provide enough information to permit the reader to judge the scope and potential importance of the article. Words in the title should convey a maximum amount of information and identify the nature of the research, organism used, and where appropriate, the technical approach (e.g. X ray, chromatography, mathematical analysis). Titles should not begin with a numeral or introductory prepositions such as "On" or "Towards" or expressions such as "A contribution to . . ." or "Investigations on . . ." Good titles greatly assist scientists and librarians in using scientific literature and aid indexers preparing titles for keyword indexes.

Abstract

The abstract should succinctly mirror the content and emphasis of the paper in about 175 words. Like the title, the abstract enables readers to determine the paper's content and decide whether or not they need to read the entire article. Begin the abstract with the main conclusion from the study, and support it with the relevant findings. Limit details of methods to those needed in understanding what was done, and work them into statements of findings. Avoid using phrases such as ". . . is discussed" or ". . . was found"; be specific. As the abstract is often divorced from the main body of the paper by abstracting and indexing services and is the only part of a paper some readers ever see, it is important that it accurately reflect the paper's contents.

Introduction

Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

Materials and Methods

Materials and Methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as "To determine . . . we . . ." If the section is long, consider using subheadings corresponding to headings for the findings.

Results

Limit the results to answers to the questions posed in the purpose of the work, and digest them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made and so avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the titles. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

Discussion

Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straightforward, it can be combined with the results section.

Acknowledgments

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

References

References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence. Nearly every manuscript submitted to the *Journal* contains errors in the references.

Tables

Tables are used to present repetitive data and should be as economical of space as possible.

Design tables to fit a 1-, 1½-, or 2-column width of the *Journal*.

Type each on a separate page and number with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Table captions should be succinct and identify the purpose of the table sufficiently well to allow the table to stand on its own.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines.

Figures

Provide photographic reproductions or the original artwork (no larger than 21.5 × 28 cm) of each illustration. If submitting photographs, type the author's name and figure name on a strip of paper and fasten to the back of the print. Do not write on the illustration itself. Provide two photocopies of line illustrations and two sets of any photographs for review purposes.

Design figures to fit a 1-, 1½-, or 2-column width of the *Journal* and ensure that all lettering, numerals, and symbols are legible, neat, and at least 1.5 mm high when reduced. Figures should not be mounted, stapled, or clipped. When possible, figures should be grouped and be done with the same or similar type of lettering.

Type legends in succession on a separate page. Add bar scales to maps, photomicrographs, equipment diagrams, etc.

Where each figure is first referred to, type "Fig. — near here."

Computer-generated figures should be avoided if possible, as they usually reproduce poorly. Dot-matrix printers cannot produce sufficiently dark, solid lines to ensure legibility. Figures drafted in black india ink are always preferable. If sheets of printed characters are used, be sure they adhere completely.

Draft rather than type axis labels for graphs.

Color plates are very expensive to produce and should only be used where the color being presented is essential to the understanding of the text. Extra costs for the reproduction of color plates are usually charged to the author. Submit only the most outstanding photographs for color reproduction. We prefer to use color photographs, but will accept drawings, color negatives, slides, or film positives.

Mathematical Expressions, Equations, and Formulae

Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulae. In the various expressions, place each component in its correct position.

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the number *one*, and between the capital *O* and the numeral *zero*. Make other explanatory notes similarly.

Appendix

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel	Bq
calorie	cal
centimetre	cm
centimetre, square	$\mathrm{cm^2}$
centimetre, cubic	$\mathrm{cm^3}$
centimetres per gram per second	$\mathrm{cm \cdot g^{-1} \cdot s^{-1}}$
coulomb	C
day	d
decimetre	dm
degree Celsius	°C
degrees of freedom	df
gram	g
hectare	ha
hertz	Hz
hour	h
joule	J
kilometre	km
litre	L
lumen	lm
lux	lx
metre	m
metre, square	$\mathrm{m^2}$
metre, cubic	$\mathrm{m^3}$
micrometre	μm
milligram	mg
millilitre	mL
millimetre	mm
millimetre, square	$\mathrm{mm^2}$
minute	min
molar mass	M
mole	mol
moles per litre	mol/L, M
month	mo
Pascal	Pa
second	s
standard deviation	SD
standard error	SE
tonne	t
volt	V
volume	vol
watt	W
week	wk
year	yr

Dates

Dates may be written in the sequence day-month-year without internal punctuation (On 9 October 1983 the . . .), or the alternative sequence month-day-year may be employed with the year set off by commas before and after it (On October 9, 1983, the . . .).

Reference Citations in Text

Name-and-year system

The *Journal* uses the name-and-year system of citation; that is, the surname of the author(s) and the year of publication are inserted in the text at an appropriate point: "Brown (1983) compared . . ." or ". . . were compared (Brown 1983)." If the reference has more than two authors, include only the surname of the first author followed by "et al." (not italicized): "Brown et al. (1983) compared . . ." or ". . . were compared (Brown et al. 1983)."

Personal communications

Personal communications are not listed in the reference list. Using parentheses in the text, state the name and mailing address of the communicator followed by "pers. comm."

Unpublished data

If the unpublished book or article has been accepted for publication, list it in the reference list section followed by the notation "(In press)". Only those manuscripts that are in galley or page proof or for which there is an acceptance letter can be considered in press. If the article is submitted but not yet accepted, state the name and address of the author of the unpublished material followed by the notation "unpubl. data" in the text and do not include it in the reference list.

Reference Lists

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *Serial Sources for the BIOSIS Data Base*) for references.

Journal article

Peterman, R. M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. 39: 1444–1452.

Entire issue of journal

Gordon, D. C. Jr., and A. S. Hourston [ed.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1): 1–365.

Book in a series

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

Book not in a series

LeBlond, P. H., and L. A. Mysak. 1978. Waves in the ocean. Elsevier, New York, NY. 602 p.

Part of book

Healey, M. C. 1980. The ecology of juvenile salmon in Georgia Strait, British Columbia, p. 203–229. *In* W. J. Neil and D. C. Himsworth [ed.] Salmonid ecosystems of the North Pacific. Oregon State University Press, Corvallis, OR.

Corporate author

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, DC. 1193 p.

Theses

Kutty, M. N. 1966. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, Univ. Toronto, Toronto, Ont. 102 p. Natl. Libr. Can., Can. Theses Microfilm No. 646.

Reports

Smith, J. E. 1981. Catch and effort statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can. Tech. Rep. Fish. Aquat. Sci. 1032: 90 p.

Translation

Koike, A., and M. Ogura. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64: 1–11. (Transl. from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983)

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h," "min," and "s" are not used, since they are the symbols for hour, minute, and second in the sense of duration or the length of time. Thus "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

Word List

The spelling of the following words is frequently inconsistent in submitted manuscripts. We prefer that authors adhere to the *Journal's* house style for these commonly used terms:

age-class (n.)
age-group (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
chi-square (n., adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
short term (n.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t-test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., adj.)

Guide des auteurs

Introduction

Les manuscrits comportant une information nouvelle dans le domaine des sciences halieutiques et aquatiques sont les bienvenus. Ils peuvent se rapporter aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influencent les systèmes de production aquatiques, et couvrir de nombreuses disciplines, y compris biologie et écologie des organismes marins et dulçaquicoles, physiologie, toxicologie, génétique, limnologie, océanographie, économique, maladies et gestion.

Les critères de sélection des manuscrits dépendent de l'étendue et de l'importance des connaissances ou idées nouvelles qu'ils contiennent. On donnera la préférence à ceux dont les résultats expérimentaux font ressortir les mécanismes sousjacents aux phénomènes observés plutôt qu'une simple description ou énoncé de fait. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles en faisant plus que corroborer des faits.

Les manuscrits soumis devraient être aussi complets que possible; des fragments de recherche sont généralement inacceptables. S'il est impossible de produire un seul article, on devra soumettre ensemble des articles étroitement liés entre eux, avec références croisées. L'article ne doit pas avoir été soumis ou accepté pour publication ailleurs.

Le *Journal* considère les types de contributions suivants :

Articles : études d'une portée générale, constituant des contributions inédites à la science.

Critiques : appréciations critiques détaillées de vastes champs de recherche, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives : essais portant sur une opinion ou une hypothèse relative aux sciences aquatiques qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans une style simple.

Commentaires : opinions sur des sujets récemment exposés dans le *Journal*, et, de préférence, ne dépassant pas 500 mots.

Soumission des manuscrits

Les manuscrits doivent être soumis au rédacteur en triplicata, et accompagnés d'une lettre mentionnant : 1) les principaux points et l'importance du travail, 2) l'acceptation des frais de publication obligatoires de 44 $ par page, 3) des suggestions quant aux arbitres éventuels et 4) une mention d'autres manuscrits contenant une information identique ou qui se rapproche de celle contenue dans le manuscrit soumis. L'auteur devra, de préférence, conserver les originaux du texte dactylographié et des figures (sauf les simili) jusqu'à ce qu'on les lui demande.

Un manuscrit est normalement soumis à deux arbitres pour évaluation, avant l'évaluation finale par un rédacteur. Cependant, les manuscrits qui ne répondent pas à la politique éditoriale ou à la portée du *Journal* sont retournés à l'auteur sans avoir été soumis à l'arbitrage.

Dispositions générales

Le *Council of Biology Editors Style Manual* (5^e éd., CBE, 9650 Rockville Pike, Bethesda, MD 20814) sert de guide quant à la forme de la présentation. Le *Webster's Third International Dictionary* ou l'*Oxford English Dictionary*, et le *Grand Larousse encyclopédique* font autorité pour l'orthographe des mots. L'uniformité de cette dernière est la responsabilité des auteurs. Les titres de périodiques sont abrégés suivant les *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103). C'est la responsabilité des auteurs de s'assurer que leurs références sont complètes et exactes. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (4^e éd. 1980. Spec. Publ. No. 12, American Fisheries Society). Pour la nomenclature génétique pour les locus de codage des protéines, le *Journal* suit les recommandations contenues dans Shaklee *et al.* (1990. Trans. Am. Fish. Soc. 119 : 2–15). Toutes les mesures doivent être conformes au système métrique et, dans la plupart des cas, être exprimées en unités SI (sauf exceptions mentionnées dans un éditorial du *Journal*, vol. 40, n^o 12), et se conformer au *Guide canadien de familiarisation au système métrique* CAN3-Z234.1-79 (l'Association canadienne de normalisation, 178, boul. Rexdale, Rexdale (Ont.) M9W 1R3).

On devra éviter les titres en séries. Un résumé doit accompagner chaque texte et exposer brièvement, en 175 mots ou moins, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé.

L'auteur doit revoir les épreuves qu'on lui remet et en corriger les erreurs typographiques. *Il doit éviter de modifier le contenu du texte.*

Cinquante tirés à part sont fournis gratuitement. Des tirés à part supplémentaires peuvent être achetés au moment de la publication (un bon de commande à cet affet accompagne les épreuves).

Préparation des manuscrits

Directives générales

La marche des manuscrits à travers les diverses étapes de l'arbitrage et de la révision sera grandement facilitée si les articles sont préparés dans le style et le format du *Journal*. En cas de doute sur des points particuliers, veuillez consulter une livraison récente du *Journal*. On trouvera ci-dessous des directives générales.

Dactylographiez le manuscrit sur papier blanc, mesurant de préférence 21,5 sur 28 cm (8,5 sur 11 pouces), sur un côté de la page. Laissez toujours une marge d'au moins 2,5 cm.

Sur la première page, dactylographiez le titre, les noms et affiliations des auteurs et les renvois qui s'y rapportent. Numérotez toutes les pages, à commencer par la page-titre, de même que celles contenant les tableaux et les légendes des illustrations.

Tout le manuscrit, y compris tableaux, légendes des tableaux et des figures, renvois et liste de références, doit être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique.

En aucun droit du manuscrit n'écrivez entièrement en majuscules.

Pour les références citées dans le texte, utilisez le système nom et année.

Liste de contrôle pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes aux buts de l'étude ?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons ? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou à l'intérieur du texte ? Est-ce que certaines données devraient être présentées séparément dans un rapport manuscrit ou dans un dépôt de données ?

Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la recherche ? Dans des énoncés de probabilité, seuls les tests statistiques sont-ils cités, et les tableaux statistiques inutiles sont-ils exclus ?

Le texte y gagnerait-il en clarté s'il plus condensé, en tout ou en partie ? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité ? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction ?

Le *titre* est-il représentatif du contenu du rapport ? Le *résumé donne-t-il l'essentiel des connaissances nouvelles ? L'introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude ? La revue de la littérature se limite-t-elle à la définition du problème ? La section *matériel et méthodes* contient-elle le minimum de détail qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données ? Les généralisations sur les *résultats* sont-elles adéquates ? Permettent-elles de distinguer entre constations et déductions ? La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes ?

Sont énumérés ci-dessous des façons d'éviter les problèmes techniques les plus communs dans la soumission des manuscrits. En accordant une attention particulière à ces points lors de la préparation de leurs manuscrits, les auteurs peuvent accélérer le traitement des articles.

1) Insérez la bande bibliographique avant le résumé (auteurs, date, titre, Can. J. Fish. Aquat. Sci.)

2) Limitez le résumé à un paragraphe d'environ 175 mots.

3) Dactylographiez à double interligne tous les éléments du manuscrit, y compris les références et les légendes des tableaux et des figures.

4) Évitez partout l'emploi exclusif de majuscules, y compris dans les en-têtes et les légendes des tableaux et des figures.

5) Ne soulignez que les noms latins d'organismes et certaines notations statistiques et mathématiques, qui devront être imprimés en italique.

6) Dans les figures, les tableaux, et le teste, exprimez les unités de mesure par leurs symboles SI propres. Pour les nombres inférieurs à l'unité, placez un zéro avant la virgule décimale.

7) Évitez les formes ambiguës, telles que $g\ C/m^2/d$; utilisez plutôt $g\ C{\cdot}m^{-2}{\cdot}d^{-1}$.

8) Identifiez le test qui a servi à évaluer la signification statistique, donnant également la valeur de probabilité. S'il s'agit de tests communs, il n'est pas nécessaire d'en donner la référence.

9) Dans le texte, généralisez à partir des tableaux et des figures ; évitez une répétition de tous les détails. Assurez-vous que tous les tableaux et figures sont indépendants et que, dans le texte, on y réfère dans l'ordre numérique. Les légendes doivent indiquer le but du tableau ou de la figure.

10) Préparez la section des remerciements au stade du manuscrit et non quand vous parviennent les épreuves.

11) En référant à des communications personnelles, donnez les initiales et l'adresse postale de la personne en cause.

12) Éliminez toutes références inutiles qui ne s'appliquent pas directement au problème.

13) Vérifiez soigneusement les références en regard des citations dans le texte et vice versa, afin de vous assurer qu'il y a correspondance. Dans le cas de matériel difficilement accessible, indiquez l'endroit où le trouver, p. ex., disponible auprès du Département d'économique, Université Simon Fraser, Burnaby (C.-B.) V5A 1S6.

14) Éliminez les virgules entre le nom et la date dans les citations telles que (Smith 1900) et soulignez *et al.*

15) Dans les en-têtes de tableaux et les axes des figures, remplacez 10^n par les préfixes SI appropriés ou utilisez des mots en clair (p. ex. milliers).

16) Aux fins de la révision, les photocopies des figures, sauf les similis, sont appropriées. Sur demande, lorsqu'il s'agira de publier l'article, envoyez des photographies des figures, ou encore les originaux, s'ils ne dépassent pas 21,5 sur 38 cm. Utilisez autant que possible un lettrage uniforme.

Parties du manuscrit

Organisation

Organisez le manuscrit par rapport au but ou à la portée de l'étude, tels qu'énoncés dans l'introduction. Il est particulièrement important que le titre et les sous-titres soient en harmonie avec les objectifs.

Avant de commencer la rédaction du manuscrit, dressez une liste provisoire des sous-titres ou en-têtes, avec le moins de divisions possible. Retouchez-les jusqu'à ce qu'ils semblent assurer, pour le lecteur, une marche logique ; ordinairement, l'ordre chronologique ne sera pas satisfaisant. Il sera plus facile d'évaluer les résultats si vous décrivez méthodes, résultats et discussion dans des sections séparées.

Organisez les tableaux et les figures de façon à faciliter les comparaisons, groupant les données apparentées en aussi peu de tableaux et de figures que possible. Assurez-vous que ces derniers sont aussi clairs que possible sans référence au texte.

Commencez les sections et les paragraphes par des énoncés généraux qui aboutissent naturellement à des énoncés particuliers. Si vous donnez d'abord une conclusion que vous appuyez ensuite, non seulement votre travail sera plus facile à lire, mais aussi les autres scientifiques pourront plus aisément en évaluer les résultats. Dans la rédaction des manuscrits, le défaut le plus commun est d'omettre de donner, au début, les généralités les plus dignes de mention.

Voyez à ce que le matériel de chaque section soit pertinent à l'en-tête et que le matériel de chaque paragraphe se rapporte à la première phrase introduisant le sujet.

Avant de rédiger les paragraphes, composez la phrase-thème de chaque paragraphe et disposez ces phrases dans l'ordre approprié.

Titre

Limitez le titre à ce que le travail veut démontrer. C'est la clé de l'article, et il devrait montrer clairement et de façon concise ce qui est contenu dans le travail lui-même. Le titre a deux fonctions : 1) il permet au lecteur de juger si, oui ou non, l'article peut avoir un intérêt et 2) il devrait fournir suffisamment d'information pour permettre au lecteur de juger de la portée et de l'importance possible de l'article. Les mots du titre devraient fournir un maximum d'information et identifier la nature de la recherche, de l'organisme utilisé et, au besoin, la méthode d'approche technique (p. ex. rayons X, chromatographie, analyse mathématique). Les titres ne devraient pas commencer par un chiffre ou par des prépositions d'introduction telles que «Sur» ou «Vers», ou des expressions telles que «Contribution à . . .» ou «Recherche sur . . .». De bons titres facilitent le classement des publications et leur utilisation par les scientifiques et les bibliothécaires.

Résumé

Le résumé doit refléter succintement le contenu et les points principaux de l'article en 175 mots environ. Comme le titre, le résumé permet aux lecteurs de prendre connaissance du contenu de l'article et de décider si, oui ou non, il leur faut lire ce dernier en entier. Indiquez au début du résumé la principale conclusion de l'étude et appuyez-la par des découvertes pertinentes. Ne donnez que les détails des méthodes nécessaires à la compréhension de ce qui a été fait et insérez-les dans l'énoncé des découvertes. Évitez l'emploi de phrases telles que «. . . on y analyse» ou «. . . a été trouvé» ; soyez spécifique. Comme le résumé est souvent séparé du corps principal de l'article par les services d'analyse et de classement, et que c'est la seule partie d'un travail que certains lecteurs auront l'occasion de voir, il est important que le résumé reflète précisément le contenu du travail.

Introduction

Limitez l'introduction à la portée, au but et à la raison de l'étude. Ne mentionnez que les travaux publiés et autres informations nécessaires à la définition du problème et à la perspective du travail. Commencez avec le but ou la portée du travail, définissez ensuite le problème et ajoutez ici et là des jalons qui aideront à orienter le lecteur. En général, une introduction n'a pas besoin de dépasser 375-500 mots.

Matériel et méthodes

Cette section renferme ce dont on a besoin pour trouver réponse aux questions soulevées dans les objectifs du travail.

Limitez les renseignements à ce qui est nécessaire pour juger de la validité des découvertes. Cette évaluation sera facilitée si vous présentez, autant que possible, toute l'information dans une section. S'il s'agit d'outillage ou de techniques déjà publiés, référez à ces publications et ne décrivez en détail que les adaptations. Il y a souvent avantage à commencer un énoncé des méthodes par une phrase qui en indique le but, comme : «Afin de déterminer . . . nous . . .». Si la section est longue, songez à utiliser des sous-titres correspondant à ceux que vous utilisez dans la section des résultats.

Résultats

Limitez les résultats aux réponses apportées aux questions en rapport avec le but de l'étude ; que ces réponses soient aussi complètes que possibles, tout en étant concises. Exprimez ce que vous avez trouvé, autant que possible dans les mêmes termes que ceux dans lesquels les observations ou les mesures ont été faites, évitant ainsi de confondre faits et spéculations. En dégageant les généralités des tableaux et des figures, référez-y et évitez de répéter dans le texte ce que les légendres devraient exprimer clairement. Le matériel complémentaire au texte peut être classé dans les séries des rapports ou dans un dépôt de données reconnu, et on y référera dans le texte.

Discussion

Limitez la discussion à l'énoncé de la principale contribution de l'étude et à l'interprétation de découvertes particulières, les comparant avec celles d'autres auteurs. On continuera à mettre l'accent sur la synthèse et l'interprétation, ainsi que sur l'exposition de généralisations et de principes largement applicables. S'il s'agit d'exceptions ou de points douteux, notez-les et faites ressortir la manière dont vos résultats corroborent ou contredisent les travaux déjà publiés. Limitez la spéculation à ce que vous pouvez appuyer sur des preuves raisonnables. Terminez cette section par un court résumé de l'importance du travail et des conclusions auxquelles il a conduit. Si la discussion est brève et précise, elle peut être combinée avec les résultats en une seule et même section.

Remerciements

Nous recommandons fortement aux auteurs de limiter les remerciements aux personnes qui ont contribué d'une manière substantielle aux aspects scientifiques et techniques de l'article, fourni une aide financière ou amélioré la qualité de la présentation. Évitez de mentionner les personnes qui n'ont fourni qu'un travail de soutien.

Références

On choisira les références avec soin, les limitant aux publications importantes. On ne peut citer dans cette section les références à des données inédites, manuscrits en préparation ou soumis à d'autres périodiques, états des travaux et communications inédites présentées à des réunions annuelles. On peut toutefois les mentionner dans le texte à titre de données inédites ou de communications personnelles (incluant les adresses). Si l'on doit citer des rapports d'experts-conseils ou d'autres documents dont la distribution est limitée, on devra préciser l'endroit où l'on peut se les procurer. On doit s'assurer que les références mentionnées dans le texte correspondent à celles qui sont contenues dans la liste de références et vice versa. Presque tous les manuscrits soumis au *Journal* contiennent des erreurs dans cette section.

Tableaux

Les tableaux sont utilisés pour présenter des données qui se répètent et devraient occuper le moins d'espace possible.

Agencez les tableaux de façon qu'ils s'adaptent soit à 1, 1½- ou 2 colonnes du *Journal*.

Dactylographiez chaque tableau sur une page séparée et numérotez-les en chiffres arabes.

Tracez des lignes horizontales de part et d'autre des en-têtes et au-dessous des colonnes, mais rarement ailleurs. N'utilisez jamais de lignes verticales. Laissez plutôt des espaces supplémentaires.

Chaque tableau doit avoir une légende instructive, complète et indépendante.

Les renvois des tableaux doivent être indiqués par des petites lettres suscrites dactylographiées au bas du tableau.

Placez les tableaux après la liste des références.

Quand on réfère à un tableau pour la première fois, on indique dans la marge du manuscrit «Tableau — près d'ici».

Notez que les *tableaux dans le texte* ne sont pas numérotés et sont dactylographiés dans le corps du texte. Ils ont rarement besoin de lignes horizontales.

Figures

Fournissez des photographies ou les dessins originaux (pourvu qu'ils ne dépassent pas 21,5 sur 28 cm) de chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas sur l'illustration même. Transmettez deux photocopies des dessins au trait et deux séries de photos qui serviront au moment de la révision.

Les dessins devront occuper une largeur de 1, 1½- ou 2 colonnes du *Journal*. Assurez-vous que tous les chiffres, lettres et symboles sont lisibles, clairs et mesurent au moins 1,5 mm de hauteur une fois réduits. Les figures ne devraient pas être montées, brochées ou fixées à l'aide de trombones. Autant que possible, elle devraient être groupées et préparées avec le même type de lettrage.

Dactylographiez les légendes, dans l'ordre, sur des feuilles séparées. Dans le cas de cartes, photomicrographies, diagrammes d'instruments, etc., ajoutez une échelle.

À l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge «Fig. — près d'ici».

On devra autant que possible éviter les illustrations préparées par ordinateur, qui ne se reproduisent pas très bien. Les imprimantes par points ne peuvent produire de lignes solides suffisamment foncées pour être lisibles. On obtiendra toujours de meilleurs résultats en utilisant de l'encre de Chine noire. Si vous utilisez du lettrage instantané, veillez à ce qu'il adhère parfaitement.

Il vaut mieux dessiner que dactylographier les inscriptions relatives aux axes.

La production de planches en couleurs est très dispendieuse et on ne devrait utiliser celles-ci que lorsque les couleurs sont essentielles à la compréhension du texte. Les coûts supplémentaires de reproduction de planches en couleurs sont ordinairement débités à l'auteur. Pour les reproductions en couleurs, ne soumettez que des photos d'excellente qualité. Nous préférons utiliser des photographies en couleur, mais acceptons dessins, négatifs, diapositives ou positifs de films en couleurs.

Expressions, équations et formules mathématiques

Laissez trois espaces au-dessus et au-dessous des lignes portant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous des équations et des formules. Placez chaque composante des différentes expressions dans la position qui lui est propre.

Indiquez clairement tous les symboles. Épelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Appendice

Abréviations

N'abrégez les termes désignant les unités de poids et mesures dans le texte que lorsqu'ils sont précédés d'un nombre.

an	an
année	a
becquerel	Bq
calorie	cal
centimètre	cm
centimètre carré	cm^2
centimètre cube	cm^3
centimètres par gamme par seconde	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
décimètre	dm
degré Celsius	°C
degrés de liberté	dl
écart type	ET
électronvolt	eV
erreur type	ErT
farad	F
gramme	g
hectare	ha
hertz	Hz
heure	h
joule	J
jour	d
kilomètre	km
litre	L
lumen	lm
lux	lx
mètre	m
mètre carré	m^2
mètre cube	m^3
micromètre	µm
milligramme	mg
millilitre	mL
millimètre	mm
millimètre carré	mm^2
minute	min
mois	mo
masse molaire	M
mole	mol
moles par litre	mol/L, M
pascal	Pa
seconde	s
semaine	sem
tonne	t
volt	V
volume	vol
watt	W

Dates

On écrira les dates dans l'ordre jour-mois-année, p.ex. 9 octobre 1983, sans virgule entre le mois et l'année.

Citations de références dans le texte

Système de nom et année

Le *Journal* utilise le système de nom et année, c.-à-d. que le nom de l'auteur ou des auteurs et l'année de publication sont insérés dans le texte à un endroit approprié : «Brown (1983) compare . . » ou «. . . on a comparé (Brown 1983)». Si la référence comporte plus de deux auteurs, inscrivez le nom du premier auteur seulement, suivi de «*et al.*» : «Brown *et al.* (1983) comparent . . .» ou «. . . on a comparé (Brown *et al.* 1983)».

Communications personnelles

Les communications personnelles ne doivent pas être incluses dans la liste des références. Utilisez plutôt des parenthèses dans le texte, entre lesquelles vous indiquerez le nom et l'adresse de la personne en question, suivis de «comm. pers.».

Données inédites

Si le livre ou l'article inédits ont été acceptés pour publication, insérez-le dans la liste des références, avec la mention «(Sous presse)». On ne peut considérer comme étant sous presse que les manuscrits au stade d'épreuves ou ceux couverts par lettre d'acceptation. Si l'article a été soumis mais n'a pas encore été accepté, ne l'incluez pas dans la liste de références, mais indiquez le nom et l'adresse de l'auteur du matériel inédit, dans le texte lui-même, avec la mention «données inédites».

Liste de références

La liste qui suit donne des exemples de ponctuation, de style et d'abréviations utilisés dans les références bibliographiques (d'après les *Serial Sources for the BIOSIS Data Base*).

Article du journal

ARCHAMBAULT, D. ET E. BOURGET. 1983. Importance du régime de dénudation sur la structure et la succession des communautés intertidales de substrat rocheux en milieux subarctique. Can. J. Fish. Aquat. Sci. 40 : 1278–1292.

Numéro complet du journal

GORDON, D. C. JR., AND A. S. HOURSTON [ÉD.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1): 1–365.

Livre dans une série

HILTON, J. W. ET S. J. SLINGER. 1981. Nutrition et alimentation de la truite arc-en-ciel. Publ. spéc. can. sci. halieut. aquat. 55 : 17 p.

Livre ne faisant pas partie d'une série

FORRESTER, W. D. 1983. Manuel canadien des marées. Ministère des Pêches et des Océans. Service hydrographique du Canada, Ottawa (Ont.). 148 p.

Partie d'un livre

AUBERT, M., J. AUBERT, S. DANIEL ET N. DESIROTTE. 1982. État actuel de la pollution bactérienne au large des côtes françaises, p. 36–40. *Dans* M. Ruivo [éd.] La pollution des mers et les ressources biologiques. Fishing News (Books) Ltd., Londres.

Corporation-auteur

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, DC. 1193 p.

Thèse

DELISLE, C. 1969. Écologie, croissance et comportement de l'éperlan du Lac Heney, Comté de Gatineau ainsi que la répartition en eau douce du Québec. Thèse doctorale, Univ. Ottawa, Ottawa (Ont.). 161 p.

Rapports

DUTIL, D. J., M. FORTIN ET Y. VIGNEAULT. 1982. L'importance des zones littorales pour les ressources halieutiques. Mémoire remis au Conseil consultatif de l'environnement du Québec. Rapp. manuscr. can. halieut. aquat. 1653 : 29 p.

Traduction

KOIKE, A., AND M. OGURA. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64: 1–11. (Transl. from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983)

Temps

On devra séparer par deux points (:) heures et minutes, et minutes et secondes. Les symboles «h,» «min» et «s» ne seront pas utilisés car ils représentent heure, minute et seconde dans le sens de durée ou intervalle de temps de douze heures et trente minutes, alors que 12:30 se rapporte à l'heure du jour.

Liste de mots

Cette section, dans la version anglaise du Guide, contient une série de mots dont l'orthographe pose certains problèmes d'uniformité, comme, par exemple, «cold water» (n.) «coldwater» (adj.); ‹hard water» (n.), «hardwater› (adj.), etc. L'auteur préparant un texte en anglais est prié de consulter la version anglaise du Guide.

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

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Ministre des Approvisionnements et Services Canada 1991

CJFSDX48(1) 1–162 (1991)
ISSN 0706-652X

Subscription rates/Abonnements

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